Universidad Autónoma de Madrid
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Islas dentro de islas: biología y conservación del paleoendemismo macaronésico *Navaea phoenicea* (Vent.) Webb & Bertel.

Memoria presentada por
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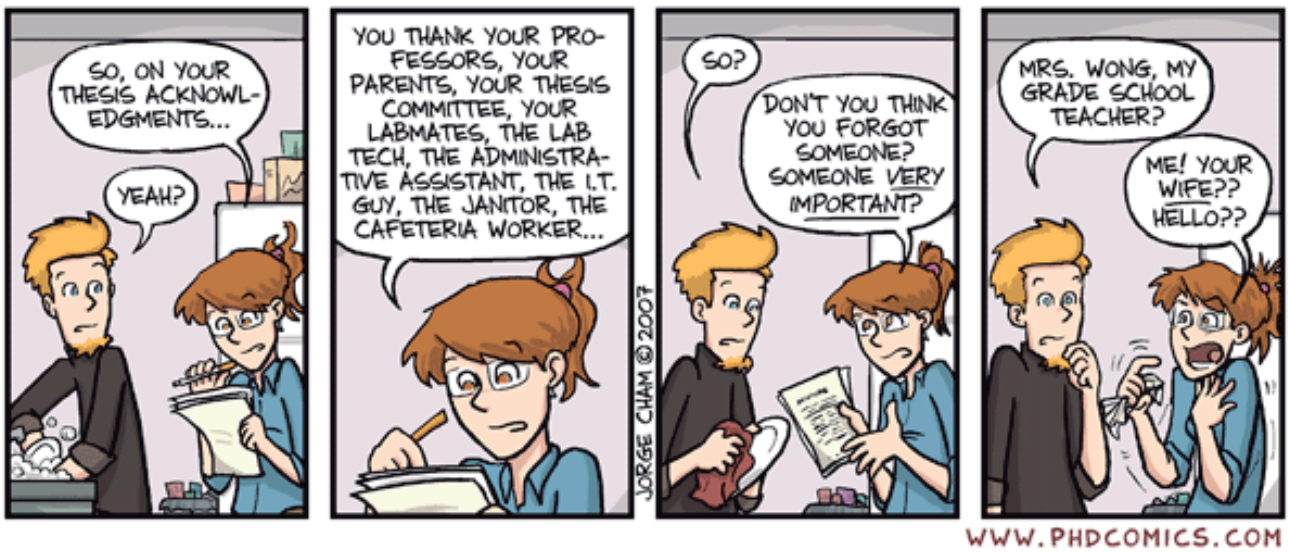

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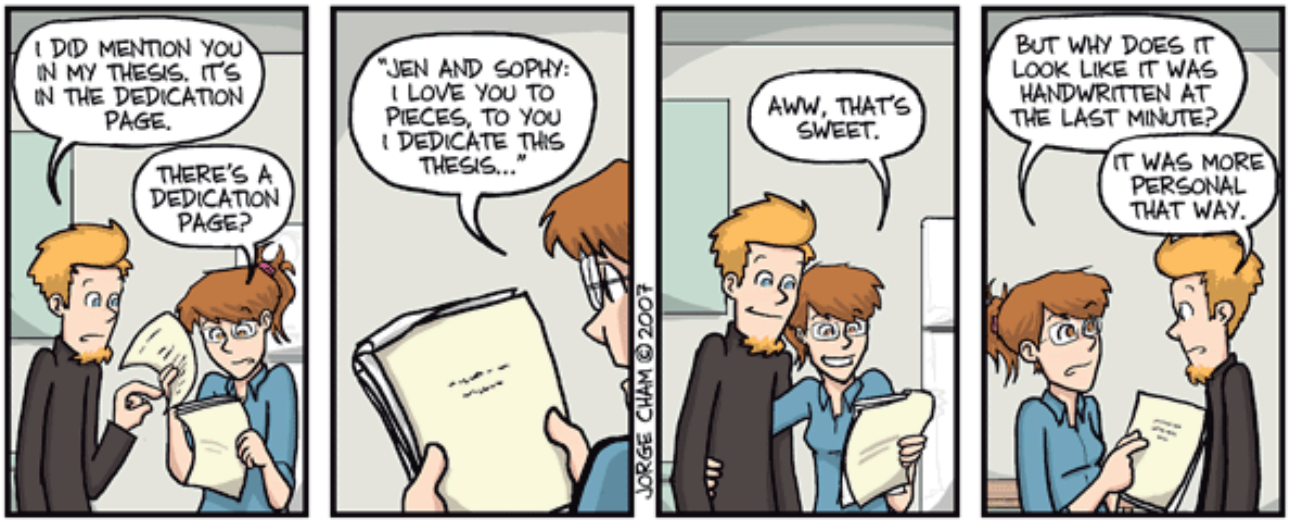

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RESUMEN

Esta tesis doctoral estudia los rasgos de la historia natural de una especie emblemática de la flora canaria, *Navaea phoenicea* (Vent.) Webb & Berthel. (Malvaceae), en un contexto ecológico y evolutivo insular. Esta especie presenta una serie de rasgos de su historia natural de gran interés científico: por un lado, se trata de un endemismo antiguo, restringido a los dos macizos montañosos más antiguos de Tenerife (paleoislas), Teno y Anaga, separados geográficamente entre sí por un área sujeta a numerosos procesos geológicos propios de las islas volcánicas. Por otro, es una especie hexaploide que es además un arbusto de gran porte, característico del fenómeno de leñosidad insular. Además, sus rasgos florales le han hecho ser incluida dentro del llamado "Elemento Ornitófilo Macaronésico", el conjunto de endemismos canarios adaptados a la polinización por aves, cuyo origen está en curso de ser esclarecido. Finalmente, es un taxon amenazado, incluido bajo sucesivas categorías de amenaza.

En este trabajo se han aplicado diversas técnicas para estudiar la filogeografía, la evolución del sistema reproductivo y la adaptación a las aves, así como las tendencias poblacionales y las causas de amenaza. La combinación de modelos de nicho ecológico de elevada resolución, pioneros para la isla de Tenerife junto al uso de AFLPs como marcadores moleculares, permite analizar los posibles escenarios de disyunción propuestos. El nicho ecológico de la especie es dependiente de parámetros climáticos relacionados con la topografía, además de confirmar su carácter rupícola. Por lo tanto su distribución disyunta parece estar ligada a los procesos geológicos acontecidos en la isla en lugar de una fragmentación causada por la actividad humana. Los marcadores moleculares revelaron por su parte unas elevadas tasas de flujo génico, tanto entre subpoblaciones como entre las dos poblaciones, mediado por la polinización por paseriformes. Bajo el escenario filogeográfico planteado, el flujo entre Teno y Anaga podría estar facilitado por posibles poblaciones intermedias ahora extintas, mientras que la geología puede aun así actuar como una gran barrera al flujo génico. Por otro lado, los valores de diversidad genética fueron, inesperadamente altos, teniendo en cuenta el tamaño tan escaso de las poblaciones.

El estudio de biología reproductiva de *Navaea phoenicea* reveló una estrategia simple por la cual el mecanismo clásico de fecundación retardada en las malváceas pierde su funcionalidad. A pesar de ser una especie completamente autocompatible, una separación temporal clara de los estadios masculino y femenino de la flor que impide la autofecundación. El resto de los parámetros medidos, como la fertilidad del polen, la receptividad de los estigmas o la ratio polen/óvulo, sí permitirían en cambio la autofecundación. Estos resultados indican una estrategia reproductiva orientada a maximizar tanto el éxito de las dos funciones masculina y femenina en detrimento del aseguramiento del éxito reproductivo mediante la autopolinización. Esta estrategia es especialmente relevante considerando que el éxito reproductivo de la especie depende por tanto de la eficiencia de los visitantes florales actuales, dado que los polinizadores originales estarían, como hipótesis principal, ya extintos. La fenología de las poblaciones es en general sincrónica, salvo en el caso de la subpoblación de Carboneras. En esta localidad, se relaciona una fenología adelantada como consecuencia de condiciones climáticas diferenciadas con una menor tasa de flujo génico con las subpoblaciones contiguas.

El análisis de la eficiencia como polinizadores de los visitantes florales reveló dos tipos de resultados. En primer lugar la alta frecuencia de visitas ilegítimas de tres especies de insectos autóctonos, incluido el abejorro canario, y de la introducida abeja melífera. Estos resultados son llamativos en tanto que las flores adaptadas a la polinización por aves suelen ser crípticas para los insectos. En segundo lugar, la eficacia como polinizadores altamente diferenciada de las cuatro especies de paseriformes visitantes: las currucas cabecinegra y capirotada presentan una mayor eficiencia sobre las parámetros del fitness masculino y femenino estimados, pero son por el contrario visitantes muy poco frecuentes. En el otro extremo el herrerillo canario es la especie que presenta una mayor frecuencia de visitas, pero sin embargo produce tasas muy pobres de fructificación de frutos debido a su comportamiento disruptor como visitante floral. Finalmente, los mosquiteros parecen los polinizadores más eficientes dado que tienen una alta tasa de visitas pero una contribución al éxito reproductivo que no es excesivamente alta. Aunque se evidencia la falta de presión selectiva de las visitas mayoritariamente ilegítimas de los herrerillos, la eficiencia como polinizadores de las demás especies no permite descartarlos como polinizadores originales salvo incorporando los datos filogenéticos y de colonización de estos paseriformes. La alta frecuencia de visitas de insectos se puede explicar por la existencia de un pico de alta reflectancia entre los espectros azul y ultravioleta en los pétalos de *N. phoenicea* y que los hace detectables por esta especie. El examen de la dinámica de producción de néctar establece varias

conclusiones: una alta secreción de néctar a lo largo de todos los estadios florales y una respuesta a la retirada de néctar de los receptáculos florales consistente en mayores secreciones. El esfuerzo de producción de néctar está diferenciado entre la fase masculina y la femenina, además de existir una asimetría espacial en la producción, coherente con la curvatura estaminal que le confiere a la flor una cierta zigomorfía. Finalmente, se hallaron células tabulares epiteliales en los pétalos, atribuidas generalmente a las flores ornitófilas como barrera mecánica a la entrada de insectos. Dado que no existen adaptaciones de color o barreras mecánicas para evitar la entrada ilegítima de insectos, la capacidad que tiene esta especie para producir tales cantidades de néctar parece adaptación suficiente para la atracción de polinizadores eficientes, sin que se haya producido una mayor convergencia de caracteres florales, a diferencia con otras especies.

El análisis de las tendencias poblacionales durante 10 años, basadas en el tamaño de los individuos, permitió establecer a partir de las matrices de transición elaboradas, modelos determinísticos y modelos estocásticos. La tasa de crecimiento finito se mostró fuertemente correlacionada con la precipitación anual en cada macizo, mientras que no se halló una evidencia suficientemente robusta sobre el efecto de vallados de exclusión de herbívoros, pese a la alta mortalidad de la clase de plántulas. Las proyecciones de los modelos apuntan a un declive de la especie modulado por la longevidad de los individuos adultos reproductores, aunque el tiempo hasta la extinción varía en gran medida entre los futuros escenarios establecidos. Sin embargo, las edades inferidas a través de los anillos de crecimiento son notablemente menores. La elasticidad de las matrices asigna el mayor peso de los cambios a los individuos reproductores. Por tanto, el principal foco de las medidas de conservación estaría puesto sobre los individuos reproductores a través de medidas de conservación del hábitat. Dadas las tendencias demográficas, inequívocamente negativas, es necesario explorar medidas complementarias. En particular, la toma en consideración del cambio climático sobre los valores de la precipitación y las posibles migraciones asistidas de la especie, y la exploración detallada del efecto de los herbívoros introducidos.

Los trabajos desarrollados en la memoria doctoral ponen en valor las respuestas generales obtenidas sobre las adaptaciones de esta especie, así como varias consideraciones metodológicas sobre el nivel de detalle y la amplitud temporal de los estudios. También la importancia de tomar en cuenta la componente espacial de los procesos en estudio. Finalmente, los resultados actuales dan una indicación de nuevas líneas de trabajo a desarrollar tanto dentro del marco evolutivo insular como el filogenético en relación a las malváceas.

Introducción

INTRODUCCIÓN

La idea de las islas como laboratorios naturales ha sido recurrente durante décadas de investigación en biogeografía, ecología, sistemática y evolución. Desde la enunciación de la Teoría sobre el equilibrio insular en Biogeografía de McArthur y Wilson (1967), los archipiélagos han sido igualmente objeto de estudio de sucesivas aportaciones a conceptos generales de la ecología y la evolución. La teoría de la selección natural de Darwin y Wallace fue concebida gracias a sus expediciones a las islas Galápagos y el archipiélago malayo, respectivamente, mientras que a Alexander van Humboldt se le reconoce la descripción de los pisos de vegetación tras su viaje a Tenerife. Con la expansión de las técnicas moleculares aplicadas a la ecología y la filogeografía, se ha revelado la composición filogenética de la biota isleña, la relación con las áreas de origen y los mecanismos de dispersión y colonización. Más allá de los patrones biogeográficos, la condición de insularidad es la causa de muchos otros patrones y fenómenos biológicos relacionados con la ecología y la evolución de las especies. Los nichos vacantes en las islas han generado cambios biológicos más generalizados en los archipiélagos conocidos como "síndromes insulares" (Whittaker & Fernández-Palacios, 2007). Los rasgos de la biota insular han facilitado por tanto el estudio de numerosas hipótesis. De manera general las islas han servido de modelo para el análisis del ciclo del taxón y las dinámicas sobre la biodiversidad (Emerson & Kolm, 2005; Whittaker *et al.*, 2007; Warren *et al.*, 2015). La relación entre los sistemas reproductivos y de dispersión en plantas han sido objeto de una discusión histórica (Baker, 1955; Stebbins, 1957; Carlquist, 1966; Lloyd, 1992; Heleno & Vargas, 2015; Vargas *et al.*, 2015), basada en la frecuencia en las islas de plantas dioicas o autocompatibles con sistemas de dispersión a larga distancia (e.g. Nogales *et al.*, 2012). Otros atributos notables son la elevada proporción de especies que muestran leñosidad insular (Whittaker & Fernández-Palacios, 2007; Lens *et al.*, 2013), y la diversidad de los endemismos de acuerdo a su origen relíctico o reciente (Cronk, 1992; Vargas, 2007). Por otro lado, los niveles de diversidad genética no están condicionados únicamente por el efecto fundador, sino por rasgos biológicos relativos al tamaño de las poblaciones, el sistema reproductivo (Stuessy *et al.*, 2013; García-Verdugo *et al.*, 2015) o la propia ontogenia de la isla (Stuessy *et al.*, 2006; Mairal *et al.*, 2015). Las redes de interacciones ecológicas, como las redes de dispersión o polinización también difieren de las continentales en cuanto a la mayor especialización de las relaciones y la menor redundancia de funciones. La menor diversidad de especies de polinizadores, especialmente de insectos por su falta de capacidad de dispersión a larga distancia, ha provocado un mayor grado de interacciones generalistas en las redes de polinización y dispersión (Kaiser-Bunbury *et al.*, 2010; González-Castro *et al.*, 2012; Trøjelsgaard & Olesen, 2013; Trøjelsgaard *et al.*, 2013; Castro-Urgal & Traveset, 2014; Traveset *et al.*, 2015). No obstante, existen diferencias entre archipiélagos en cuanto a rasgos de las redes mutualistas (Nogales *et al.*, 2015).

La explosión de biodiversidad de los ecosistemas insulares ha hecho de ellos entornos extremadamente frágiles debido a su tamaño restringido y la consecuente limitación de tamaño de las poblaciones. Otros factores que afectan a las especies insulares son las elevadas tasas de endemicidad, la intensidad de los procesos de antropización y la vulnerabilidad y exposición a vectores de cambio global como las especies invasoras o la degradación y fragmentación de los hábitats (Whittaker & Fernández-Palacios, 2007). A los factores biológicos intrínsecos y a las amenazas históricas se ha unido ahora el cambio climático, que afecta a los ecosistemas insulares de manera distinta a los continentales (Harter *et al.*, 2015).

Las Islas Canarias son uno de los archipiélagos biológicamente mejor estudiados (Whittaker & Fernández-Palacios, 2007). Su posición subtropical tan cercana geográfica y administrativamente a Europa le ha otorgado un trasfondo histórico de exploraciones naturalistas internacionales, de modo que Canarias ha sido frecuentemente la primera parada de muchas expediciones históricas europeas que se han recopilado en iniciativas como el Proyecto Humboldt (http://humboldt.mpiwg-berlin.mpg.de/01.introduccion.htm). Charles Lyell en su objetivo de convertir la geología en una ciencia moderna realizó estudios pormenorizados del vulcanismo insular; el desembarco de Alexander van Humboldt, en su viaje hacia Venezuela, supuso una de los mayores hitos en las expediciones naturalistas y permitió la descripción de fenómenos biológicos como la distribución altitudinal de la vegetación. Charles Masson, Sabin Berthelot, Étienne Pierre Ventenat, Philip Webb o Eric Sventenius fueron algunos de los naturalistas que impulsaron la taxonomía en las islas. Desafortunadamente las Islas Canarias no pudieron ser examinadas por Darwin, quien intentó desembarcar dos veces en la isla. El interés naturalista por las islas ha trascendido significativamente a toda la sociedad con

el interés por el porte del drago canario (*Dracanea draco*), el tamaño de los, muchas veces amenazados, lagartos endémicos de cada isla, la importancia paisajística de la laurisilva, o las inflorescencias de tajinastes rojos (*Echium wildpretii*) y picantes (*E. auberianum*) en un paisaje como el de las Cañadas del Teide. Las Islas Canarias son un caso de estudio paradigmático de la biogeografía insular que cumple con todos los rasgos de los sistemas isleños: combina estabilidad climática, complejidad topográfica y ecológica, diferentes edades de emergencia, distancias al continente e historias geológicas. Igualmente supone un importante reto para la conservación, ya que en el territorio operan todos los principales factores de amenaza sobre su diversidad biológica.

Dentro del archipiélago, Tenerife se caracteriza por ser la isla más compleja desde el punto de vista geológico (Ancochea, 2004) y la más diversificada en cuanto a sus hábitats debido a su mayor tamaño, gradiente de altitud y contrastes climáticos. Desde una perspectiva biogeográfica evolutiva, la isla funciona como un centro de diversificación entre las islas más jóvenes y alejadas del continente y las más antiguas, erosionadas y cercanas. Alberga además parte de los principales centros de endemismos de Canarias, siendo el macizo de Anaga en el extremo este el área más rica en endemismos de todo el área administrativa europea (Reyes-Betancort *et al.*, 2008; Carine *et al.*, 2009). En total, Tenerife cuenta con las mayores tasas de biodiversidad de Canarias (Fernández-Palacios & Whittaker, 2008): 823 de 2371 endemismos de una sola isla, 2031 endemismos sobre 3672 en todas las islas, y 8853 especies en total sobre 13328 que cuenta todo el archipiélago.

Especie de estudio: *Navaea phoenicea* (Vent.) Webb & Berthel.

Esta tesis utiliza como caso de estudio un endemismo de la isla de Tenerife, la especie *Navaea phoenicea* (Vent.) Webb & Berthe. (Malvaceae), llamada localmente malvarrisco, higuereta, o malva de risco encarnada (figura 1). Su elección como objeto de estudio está fundamentada por varias características clave de su historia natural que la hacen enormemente atractiva desde un punto de vista evolutivo, biogeográfico y conservacionista:

Su posición taxonómica, inferida mediante filogenia molecular (Tate *et al.*, 2005; Escobar García *et al.*, 2009), emplaza a la especie dentro de una rama hermana al linaje de los géneros *Lavatera* y *Malva*, dentro la tribu Malveae, subtribu Malvineae inscritos en la denominada alianza genérica de *Malva* (Bates, 1968; Bates & Blanchard, 1970). Esta divergencia sugiere un origen previo a la diversificación de la alianza genérica de *Malva* en las distintas regiones de clima mediterráneo del mundo.

Como la mayoría de la especies de su linaje, se trata de un taxon poliploide, concretamente hexaploide (2n= 48). Llama la atención por tratarse de un endemismo insular canario donde no es frecuente la presencia de poliploides (Borgen, 1979). La especie ha desarrollado un biotipo arbustivo, que la encuadra en su linaje como ejemplo de leñosidad insular. Este hecho es igualmente compartido con la otra malvácea endémica existente en Canarias, *Lavatera acerifolia*, presente en todas las islas excepto El Hierro. Ambas especies, aunque comparten el porte arbustivo, aunque menor que el de la higuerete y su naturaleza hexaploide, constituyen dos colonizaciones independientes de la alianza genérica de Malva en Macaronesia. Al contrario de la posición aislada de *N.*

Figura 1. Flores de Navaea phoenicea. Curruca cabecinegra tomando néctar de las flores de *N. phoenicea*.

phoenicea, *Lavatera acerifolia* es una especie hermana de *L. maritima* de distribución mediterránea occidental, y que en Canarias habita en hábitats más xéricos que los de la higuereta.

Presenta la higuereta una distribución disyunta con dos poblaciones en los extremos de Tenerife, en los macizos de Teno (este) y Anaga (oeste), que son además las zonas más antiguas de la isla y coincide con dos centros de endemismo del archipiélago dentro de un patrón de distribución de especies generalizado (Reyes-Betancort *et al.*, 2008; Carine *et al.*, 2009). La disyunción permite trabajar con varios escenarios e hipótesis sobre las causas que afectan a la discontinuidad en la distribución, para discernir si las especies comparten las causas de la disyunción o si, por el contrario, han sufrido procesos distintos.

3) *Navaea phoenicea* se ha incluido en el conjunto de especies potencialmente ornitófilas de Macaronesia, conocido como el Elemento Ornitófilo Macaronésico. Se trata de un grupo de especies de linajes independientes adaptadas aparentemente a la polinización por aves, con caracteres florales del síndrome de polinización por aves: colores rojos, amarillos o anaranjados, grandes cantidades de néctar diluido, morfología generalmente abierta, floración invernal y ausencia de olor (Faegri & Van Der Pijl, 1979; Ollerton *et al.*, 2009). Aunque la polinización por aves es común en zonas tropicales, este mutualismo es menos frecuente en las áreas templadas, como California o Sudáfrica (Trøjelsgaard & Olesen, 2013). La aparición de un fenómeno de convergencia adaptativa en linajes independientes de distintas familias de plantas, en ausencia, al menos actualmente, de aves nectarívoras en las islas, ha suscitado un intenso interés científico (Ojeda, 2013). Actualmente el Elemento Ornitófilo Macaronésico y su potencial síndrome de polinización es objeto de estudio para elucidar las causas de la aparición de estas interacciones. En el caso de *Navaea phoenicea*, la adaptación a la ornitofilia sería consecuencia probable de su carácter relicto. Desde los trabajos originales de Vogel *et al.* (1984) y Olesen (1985), se han considerado 16 especies en 8 géneros (Ojeda, 2013)♪. El origen del Elemento Ornitófilo Macaronésico no está completamente esclarecido, pero las hipótesis enunciadas por Valido y Olesen (2004) mantienen que mientras algunas especies tendrían un síndrome antiguo de origen africano por presión selectiva de aves especialistas (hipótesis relicta), otras de origen más reciente habrían desarrollado el síndrome *de novo* en respuesta a los paseriformes oportunistas presentes en Canarias (Olesen, 1988; Rodríguez-Rodríguez & Valido, 2008, 2011; Olesen *et al.*, 2012; Ojeda *et al.*, 2013; Rodríguez-Rodríguez *et al.*, 2013).

Figura 2. Ilustración de *Navaea phoenicea* creada por Joseph Redouté para el libro de E.P. Ventenat Le Jardin de la Malmaison.

4) *Navaea phoenicea* es una especie reconocidamente amenazada, con un bajo número de efectivos y con sus factores de riesgo parcialmente identificados: escasas poblaciones, dificultad para el reclutamiento de nuevos individuos, competencia con especies invasoras y degradación del hábitat. Ha sido considerada en consecuencia bajo varias categorías de protección en Libros Rojos y Listas Rojas publicadas (Gómez-Campo, 1996; VVAA, 2000; Rodríguez Núñez *et al.*, 2004; Moreno-Saiz, 2008). No así, sin embargo, en el reconocidamente polémico catálogo de especies amenazadas del Gobierno de Canarias, que únicamente la considera "de especial interés para los ecosistemas" (Gobierno de Canarias, 2010; Fernández-Palacios & Nascimento, 2011). Es urgente por tanto hacer un seguimiento de la viabilidad poblacional de la especie además de valorar y proponer medidas de conservación. Esto es tanto más necesario considerando la singularidad evolutiva de la especie, y el papel funcional en las redes de polinización de esta especie en los ecosistemas insulares.

5) El carácter ancestral de una especie relicta, la disyunción como barrera para el flujo génico y un bajo número de efectivos, son factores que favorecen la deriva

genética y los cuellos de botella para la especie. En cambio, la polinización por aves puede favorecer el flujo génico, pero modulado en función de la tasa de autopolinización en la naturaleza o la abundancia de los polinizadores. Es preciso por tanto utilizar los marcadores moleculares para establecer el grado de endogamia de la especie así como qué factores han favorecido su aparición, además de sus implicaciones para la conservación.

La historia natural de la especie y sus implicaciones motivan la formulación de varias preguntas profundamente interrelacionadas sobre, por ejemplo, el éxito reproductivo mediado por polinizadores o por autopolinización, su influencia en la estabilidad demográfica y en las tasas de endogamia, y las estrategias de conservación de la especie. Es necesario además determinar y localizar los hábitats potenciales para entender la distribución actual pero también para la propuesta de medidas de conservación. Las relaciones entre los aspectos de la historia natural de esta especie nos han motivado a realizar un estudio integral sobre los interrogantes de esta especie: su filogeografía, su adaptación a la polinización por aves y las implicaciones para la conservación.

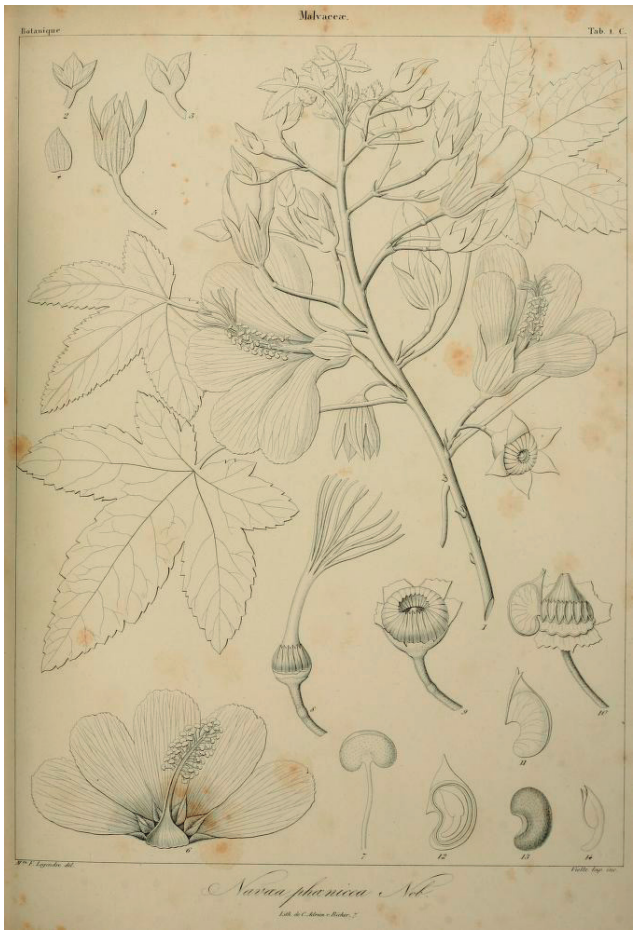


Figura 3. Ilustración de *Navaea phoenicea* de Webb & Berthelot en Histoire Naturelle des Iles Canaries.

N. phoenicea fue inicialmente denominada *Lavatera phoenicea* por Étienne Pierre Ventenat en base a la distinción taxonómica clásica del epicáliz de las malváceas (Ventenat, 1803, figura 2). La presencia de dos aurículas conformando un receptáculo en la base del pétalo para mantener grandes cantidades de néctar determinó la inclusión en un género monotípico independiente creado en honor al Marqués de Nava, fundador del Jardín de Aclimatación de La Orotava, por parte de los naturalistas Philip Webb y Sabin Berthelot (Webb *et al.*, 1840, figura 3). Los estudios moleculares sobre la tribu Malveae confirmaron el carácter independiente y basal de la especie respecto al clado *Lavatera-Malva* (Tate & Fuertes Aguilar, 2005; Escobar García *et al.*, 2009), si bien existen conflictos entre los árboles filogenéticos nucleares y cloroplásticos (Escobar García *et al.*, 2009).

Morfológicamente, se trata de un arbusto o arbolillo ramificado desde la base y de mayor porte que las especies mediterráneas, y que ha desarrollado por lo tanto arborescencia insular. Sus hojas son palmatilobuladas con pecíolos llamativamente largos. Es una especie caducifolia estival aparentemente en respuesta a la falta de agua, puesto que los ejemplares cultivados irrigados mantienen la hoja. El color de las flores está entre el naranja y el rojo oscuro. En la base de los pétalos se encuentra el llamativo receptáculo nectarífero, exclusivo de esta especie. Los rasgos florales apunta la pertenencia al conjunto de plantas macaronésicas ornitófilas tanto por el color floral como las cantidades masivas de néctar. Las flores se disponen en racimos o solitarias, y producen un fruto esquizocárpico doblemente acuminado, que contiene semillas reniformes de testa gruesa. Florece a partir de las primeras lluvias post-estivales hasta febrero.

Dentro de la distribución disyunta restringida a las paleoislas de Anaga y Teno, su hábitat principal son escarpes rocosos y barrancos en la banda altitudinal del bosque termófilo siempre en la cara norte. Eventualmente puede descender al piso del cardonal-tabaibal costero o ascender hasta encontrarse poblaciones en la banda altitudinal de la laurisilva y el fayal-brezal. Se trata por tanto, como ocurre frecuentemente con las malvas, de una especie rupícola o subrupícola, con algunos individuos en zonas antiguamente aterrazadas para agricultura, en las zonas de menor radiación solar, allá donde se puede acumular cierta cantidad de materia orgánica. La falta de accesibilidad a los barrancos ha condicionado severamente los trabajos de esta tesis, que en ocasiones han requerido técnicas de trabajo vertical y anclajes fijos puestos sobre

roca débil. La notación utilizada en esta memoria es la siguiente: el concepto de población se refiere al conjunto de los individuos en cada uno de los dos macizos. Dentro de cada uno de ellos, se denominan subpoblaciones a los pequeños núcleos de individuos agregados de cada macizo. Así, buena parte de los individuos se han descartado para realizar experimentos de campo, pudiendo así verse comprometidas las estimaciones de su tamaño muestral.

Estudios sobre biología reproductiva de *Navaea phoenicea*

- Fuera de los trabajos realizados en el contexto de esta tesis doctoral y de los trabajos moleculares sobre malveas, *Navaea phoenicea* es una especie mal conocida, si bien existen algunos trabajos que han incluido ciertos aspectos de la biología reproductiva de esta especie:
- En el plano demográfico de los proyectos de elaboración de los Libros Rojos de Flora Amenazada (Gómez-Campo, 1996; Bañares-Baudet *et al.*, 2004), recogen tanto un censo que estima un tamaño poblacional de 1400 individuos, como una exploración cualitativa de los posibles factores de riesgo para la especie. Así, entre los factores de riesgo se enumeran la herbivoría, la competencia con alóctonas, el parasitismo y la falta de capacidad dispersiva y de regeneración.
- La composición del néctar ha sido superficialmente analizada por Dupont *et al.* (2004), determinando que el azúcar del néctar de las especies ornitófilas está compuesto por monosacáridos.
- La anatomía de las células epiteliales de los pétalos del Elemento Ornitófilo Macaronésico ha sido explorado por Ojeda *et al.* (en preparación), relacionando el carácter ornitófilo de las especies estudiadas con la ausencia de células epiteliales estriadas en los pétalos.
- En el estudio del desarrollo anatómico de flores en las malváceas de Vitarelli *et al.* (en preparación), se ha identificado una cantidad significativamente mayor de tricomas nectaríferos en la especie de estudio que en el resto de las especies estudiadas (figura 4).
- Las diferencias morfológicas en el polen de diferentes especies de malvas (Pereira Coutinho *et al.*, en preparación) han permitido diferenciar un mayor tamaño del polen en las especies ornitófilas de malvas (figura 5).
- Finalmente y como trabajo posterior a los estudios desarrollados para esta memoria, como consecuencia de los hallazgos en el campo, se ha realizado un estudio que identifica al lagarto tizón *Gallotia galloti* como dispersor ocasional de las semillas (Fernández de Castro *et al.*, en preparación).

Ecología y biogeografía del archipiélago canario

Canarias (figura 6) es el archipiélago más extenso de la Región Macaronésica junto a Madeira. El sistema actual consta de 7 grandes islas (Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Palma, La Gomera y El Hierro) además de las pequeñas islas de La Graciosa, Alegranza y Lobos. Geográficamente se trata de una cadena de islas alineada en un eje Este-Oeste, a lo largo del cual las islas han ido cronológicamente emergiendo. De esta manera, las islas más antiguas en el complejo son Lanzarote y Fuerteventura, con 25 millones de años (MA), se encuentran a menos de 100 km de la costa norteafricana, mientras que El Hierro, con únicamente 1.5 MA, se encuentra a más de 300 km. La interpretación sobre el origen de las islas como sistema volcánico queda resuelto con la propuesta de un modelo unificado por Anguita y Hernán (2000). Los autores vinculan el origen del vulcanismo en el Atlas marroquí con el de Canarias, a través de un relicto de una antigua gran

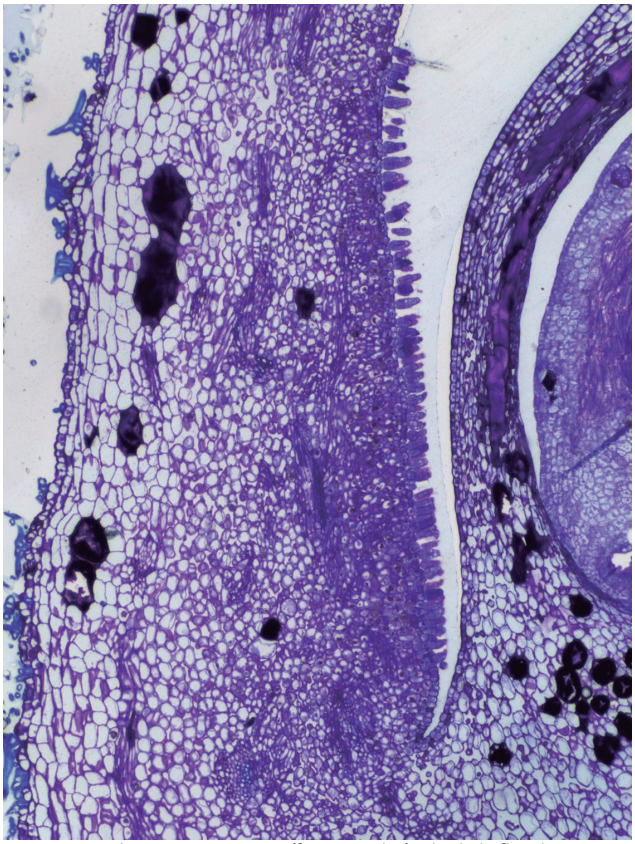


Figura 4. Tejido y tricomas nectaríforos en el sépalo de la flor de *Navaea phoenicea*.

pluma magmática presente en la parte superior del manto, que alcanza el oeste europeo. Este modelo permite explicar coherentemente las semejanzas geológicas con actividad volcánico en el norte de África y el avance irregular en la aparición de las islas, con respecto al eje de rotación de la placa africana.

Fernández-Palacios *et al.* (2011) proporcionan una revisión exhaustiva de la historia evolutiva de los sistemas insulares macaronésicos desde la aparición de las primeras paleoislas, incluyendo asimismo la subida en el nivel del mar del último máximo glacial. La diferencia de edades entre islas ha propiciado las diferencias actuales en sus estados ontogénicos (*sensu* Fernández-Palacios *et al.* 2011). Así, las islas orientales pueden considerarse maduras o senescentes, lo que se traduce en relieves topográficos planos debido a la erosión. En un estado intermedio se encuentran Tenerife y Gran Canaria. La Palma y La Gomera y, sobre todo El Hierro, son islas más jóvenes, con relieves ya mucho más elevados pero con procesos erosivos ya en marcha. Los trabajos de Báez (1992) Juan (2000) y posteriormente de Whittaker & Fernández- Palacios (2007) y Caujapé-Castells (2011) proporcionan sucesivas revisiones de los estudios biogeográficos en Canarias. Sanmartín *et al.* (2008), al realizar la primera inferencia biogeográfica de la dispersión inter islas para Canarias basada en modelos bayesianos, deducen una mayor tasa de dispersión para las islas centrales del archipiélago. Basándose en la misma metodología, se ha inferido el patrón biogeográfico las especies pertenecientes a la *Rand Flora* africana (Sanmartín *et al.*, 2010).

Tenerife representa una entidad más particular porque su historia geológica es más compleja. El estado actual de la isla es el resultado de la unión de tres islas independientes seguido por un proceso de vulcanismo intenso y grandes derrumbes masivos (Ablay & Hürlimann, 2000; Ancochea, 2004; Hürlimann *et al.*, 2004; Boulesteix *et al.*, 2012, 2013). Una descripción más extensa se encuentra en el capítulo 1 de esta memoria. Tenerife muestra por tanto unidades topográficas muy diferenciadas con implicaciones para la distribución de las especies en dos sentidos. Por un lado, las unidades geológicas configuradas a lo largo de la ontogenia de la isla tienen efectos sobre la biogeografía de muchas especies que muestran un patrón de distribución ligado o bien a las paleoislas, o bien modificado por los derrumbes, manteniendo las barreras para el flujo génico. Algunos ejemplos son las poblaciones locales de *Gallotia gallotii* en el valle de Güimar (Brown *et al.*, 2006), *Eutrichopus* (Carabidae) (Moya *et al.*, 2004) o *Canarina canariensis* (Mairal *et al.*, 2015). Por otro lado, existe una gran diversidad de hábitats que responden a las variaciones de orientación y altitud. La retención de la humedad de los vientos alisios por las montañas permite la persistencia de la laurisilva en la cara norte de la isla, mientras el pinar de *Pinus canariensis* aparece en la cara sur a la misma altitud salvo en las zonas más umbrías de los barrancos, donde se acantonan elementos de vegetación más higrófila. En zonas de alta montaña por encima del pinar se encuentra la vegetación de cumbre.

En línea con la tendencia global que acontece en las islas, el archipiélago en conjunto afronta un grave deterioro de su biodiversidad, con un 25% de las plantas en estado

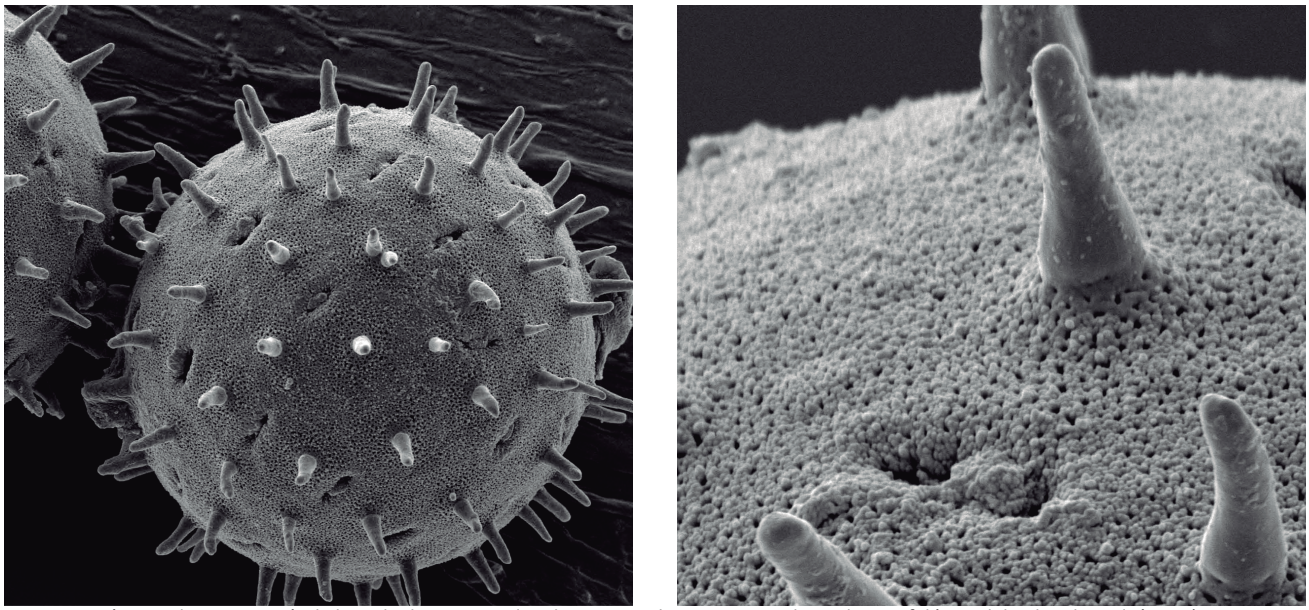


Figura 5. Imágenes de microscopía de barrido de granos polen de *Navaea phoenicea* para el estudio morfológico del polen de Malváceas (Pereira-Coutinho *et al.*, en preparación).

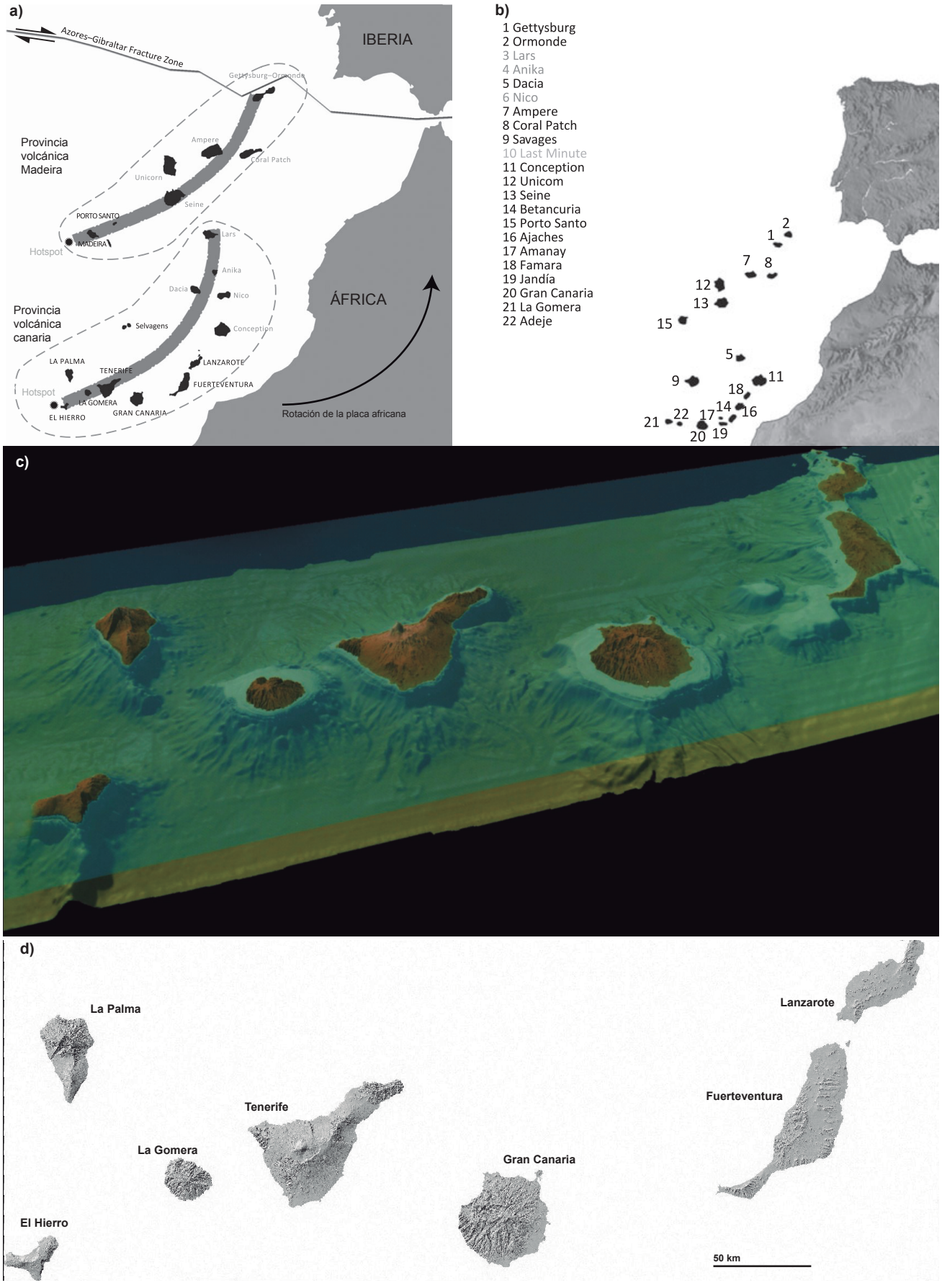


Figura 6. a) Localización geográfica de los archipiélagos Macaronésicos; b) Reconstrucción de la Macaronesia hace 10 millones de años. Modificado de Fernández-Palacios *et al.* (2011); c) Modelo Digital del Terreno tridimensional de Canarias (IGN); d) Modelo de sombras de Canarias.

de riesgo (Bañares-Baudet *et al.*, 2004). Canarias, que es considerado un *hotspot* de biodiversidad (Médail & Quézel, 1999; Machado, 2002), ha sido objeto de una alteración de los ecosistemas desde la aparición de los guanches en las islas, provocando entre otros efectos el peor estado de conservación conocido de la flora de un determinado territorio (Moreno-Saiz *et al.*, 2015). Actualmente muchos ecosistemas han sufrido profundas regresiones (del Arco Aguilar, 2006; del Arco Aguilar *et al.*, 2010). La laurisilva, como bosque emblemático en Canarias, ha sufrido un gran deterioro como en el caso de Gran Canaria, donde es prácticamente ya inexistente. A pesar de la severa perturbación, los estudios palinológicos apuntan sin embargo a que en épocas históricas también se ha visto favorecido (De Nascimento *et al.*, 2009; Fernández-Palacios *et al.*, 2011). Las zonas de vegetación xerófila costera han sido generalizadamente afectadas por la planificación urbanística, turística e industrial. La biota isleña está además afectada gravemente por la introducción de especies alóctonas, como ratas que predan sobre especies de la laurisilva, o herbívoros de pequeño y gran tamaño que han llegado a esquilmar la vegetación de áreas protegidas como las Cañadas del Teide o la Caldera de Taburiente. Los análisis de las listas rojas detectan la herbivoría y las especies exóticas como amenazas principales para las especies vegetales en Canarias y la han convertido a nivel mundial, en la más amenazada de las floras evaluadas en un territorio (Moreno-Saiz *et al.*, 2015). El cambio climático, considerada como una de las amenazas más graves a nivel global, no ha sido apenas estudiado en el ámbito Macaronésico.

El tamaño y la topografía de Tenerife la han hecho especialmente propensa a la ocupación humana. A través de los registros palinológicos se ha constatado la desaparición de especies mediterráneas de los bosques de Tenerife como los *Quercus* durante la época de los guanches (De Nascimento *et al.*, 2009). La llegada de las poblaciones

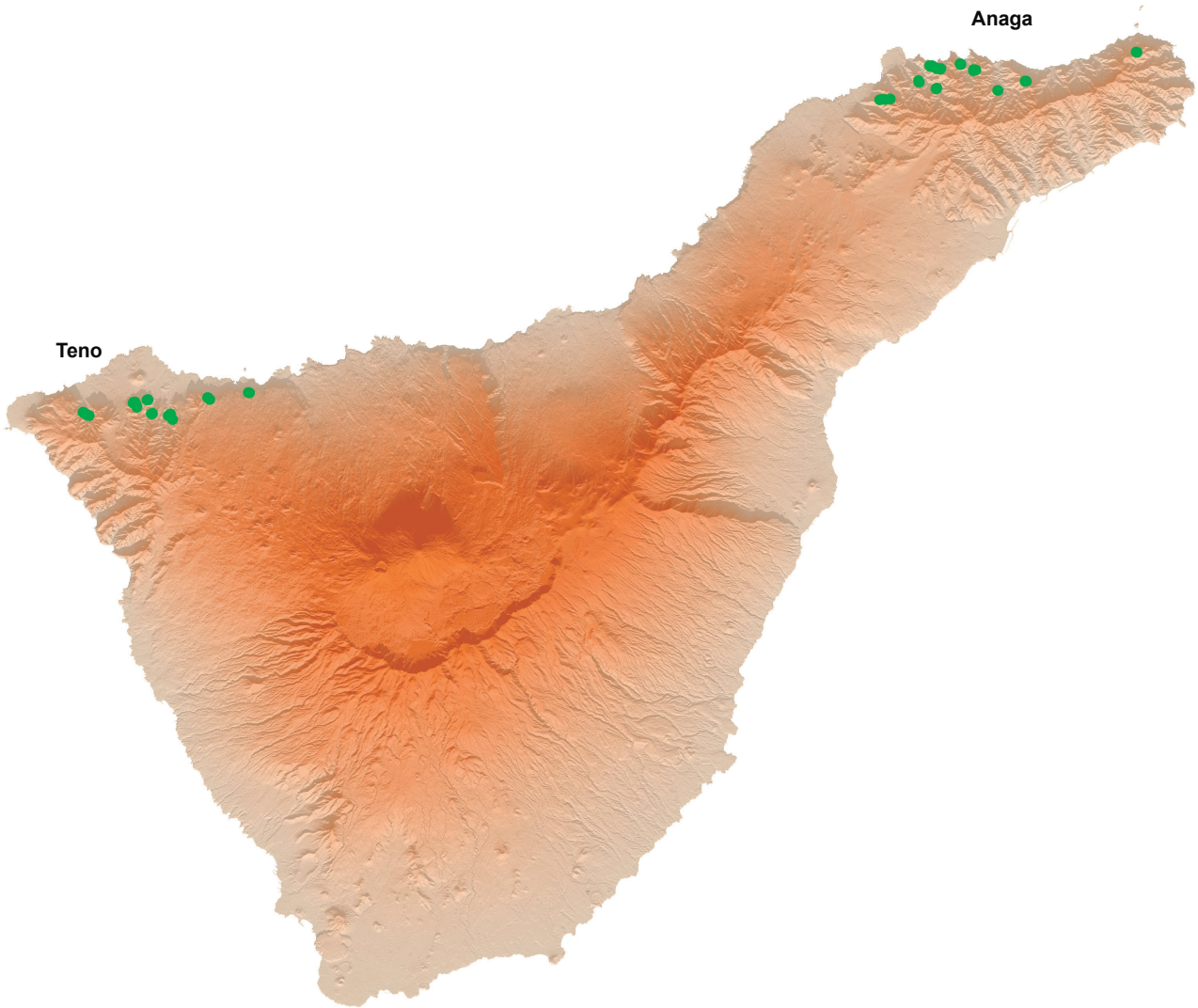


Figura 7. Modelo de sombras para la isla de Tenerife, con la localización de las subpoblaciones de *N. phoenicea* en Teno y Anaga.

europeas y la creación de una economía agrícola de exportación propiciaron la transformación de la vegetación del centro de la isla para el cultivo y la urbanización, que ha perdurado hasta la actualidad. Apenas quedan restos de vegetación natural a lo largo del centro de la cara norte. Los macizos de Teno y Anaga sin embargo han permanecido históricamente mejor conservados. El uso de la laurisilva en aquellas zonas ha sido tradicionalmente restringido para preservarla como fuente de recursos hídricos, y actualmente ambos macizos están protegidos bajo las figura de Parque Rural, y además Anaga ha sido recientemente nombrada Reserva de la Biosfera de la UNESCO. Las medianías de los macizos por el contrario han sido sometidas a laboreo muy extendido hasta mediados del siglo XX a pesar de la orografía del terreno, por lo que el bosque termófilo no ha podido empezar a recuperarse hasta que no ha habido un progresivo abandono de cultivos. Aun así, buena parte de la medianía está afectada por la invasión principalmente de *Opuntia ficus-indica* y *Opuntia dillenii* entre otras especies (del Arco Aguilar, 2006; del Arco Aguilar *et al.*, 2010).

OBJETIVOS Y CONTENIDOS

La memoria doctoral se ha estructurado en capítulos que agrupan objetivos e hipótesis similares. El propósito es ofrecer un hilo conductor que tiene como punto de partida el objetivo de esclarecer la disyunción, y a partir de los análisis que permiten su resolución, abrir el planteamiento de nuevas hipótesis y posteriores respuestas hasta tener un panorama general exhaustivo de la historia natural de la especie. Aunque los resultados de los diferentes capítulos están necesariamente relacionados entre sí, cada uno puede leerse y entenderse en gran medida por separado. La introducción de cada capítulo ahonda en los antecedentes de las cuestiones enumeradas aquí, y se exponen de manera detallada las hipótesis de trabajo:

Objetivos

1. Discernir los escenarios más plausibles de la disyunción en la distribución;
2. Estudiar los efectos de la disyunción y los polinizadores sobre la diversidad genética y el flujo génico entre poblaciones.
3. Determinar la eficacia de los visitantes florales como polinizadores y estudiar los rasgos florales de la especie para probar las hipótesis del origen de su potencial carácter ornitófilo.
4. Realizar un análisis de viabilidad poblacional para determinar su grado de amenaza y proponer medidas de conservación.

Capítulo 1

Se plantean varios escenarios no excluyentes sobre las causas de la disyunción Anaga - Teno: 1) limitación del nicho ecológico a las paleoislas; 2) destrucción de poblaciones en el centro de la isla por los derrumbes masivos de laderas en época antigua, o 3) destrucción de las poblaciones centrales por destrucción humana tras la colonización. Para mostrar las evidencias a favor de uno u otro escenario, se construyeron capas espaciales de muy alta resolución de clima y topografía inéditas para Tenerife. La información climática y topográfica, así como la distribución precisa de la especie se utilizó para modelizar la distribución potencial en ausencia de actividad humana y cuantificar la contribución de distintas variables topoclimáticas a la configuración del nicho ecológico de la especie.

Los resultados limitan la presencia de la especie a las paleoislas y a los barrancos centrales, pues la especie se revela dependiente de las condiciones climáticas condicionadas al microrrelieve. Por tanto parece más plausible un escenario que constriñe la presencia de la especie a las zonas de paleoislas que no se extiende a las

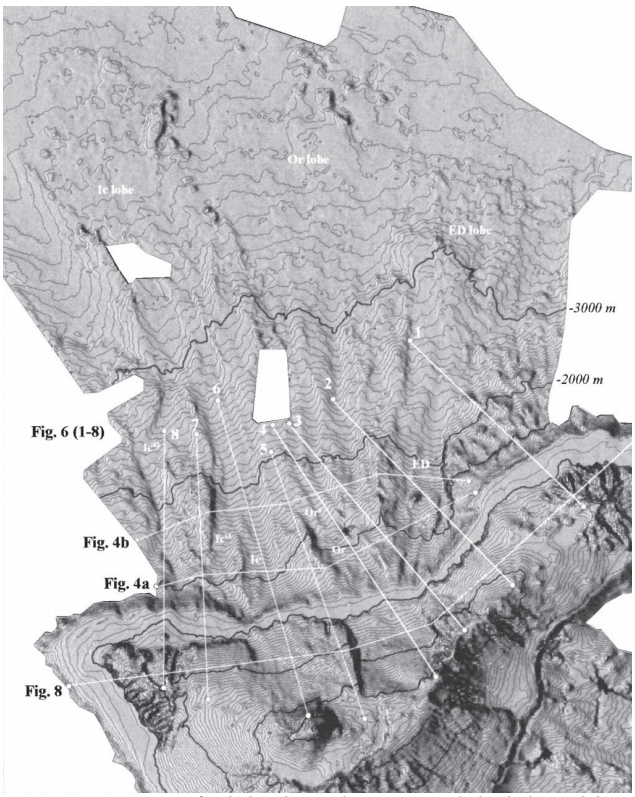


Figura 8. Reconstrucción de los derrumbes masivos de las laderas del norte de Tenerife. Modificado de Ablay & Hürlimann (2000)

zonas centrales de las islas. Podrían haber existido algunas poblaciones intermedias que podrían facilitar el flujo génico entre ambas poblaciones.

Capítulo 2

La disyunción en la distribución puede actuar junto con factores ambientales presentes en las islas para configurar la estructura y diversidad genética de las poblaciones de *Navaea phoenicea*: frente a las barreras geográficas, el carácter ancestral y el bajo número de efectivos que dificultan el flujo génico y favorecen la depresión endogámica, el tipo de polinizadores puede contrarrestar este efecto. Se emplearon marcadores moleculares dominantes (AFLPs) para establecer la estructura genética de las poblaciones y el flujo génico, a la vez que se estudia el movimiento de los paseriformes potencialmente polinizadores y su papel como vectores del flujo génico.

El análisis molecular revela una diversidad genética superior a la esperada, y a pesar de una estructura genética clara existe un elevado flujo génico entre subpoblaciones y poblaciones, con cierta introgresión entre ellas. Los patrones de movimiento de polen mediado por los polinizadores entre núcleos son coherentes con los niveles de flujo génico encontrados, lo que confirma su papel como vectores de flujo génico, evitando así la deriva genética.

Capítulo 3

La biología reproductiva de las malváceas adopta generalmente una estrategia de hercogamia junto a la fecundación retardada para asegurar en última instancia la autopolinización, cuando los estilos se revuelven hacia la columna estaminal, aunque se han discutido otras estrategias reproductivas igualmente complejas que pueden darse en malváceas. Los sistemas reproductivos son especialmente relevantes en islas, donde las tasas de autofecundación pueden más determinantes para las plantas, en términos demográficos y de diversidad genética. Por ello, y como punto de partida para entender la biología floral de *Navaea phoenicea*, se estudió su sistema reproductivo y su grado de autocompatibilidad.

En cuanto a su fenología se localizó una subpoblación con menor sincronía en la floración respecto al resto, que puede haber derivado en un cierto aislamiento genético. Respecto al sistema reproductivo, la especie es completamente autocompatible, y la fertilidad de polen y estigmas es suficiente para asegurar la autofecundación retardad. Sin embargo la especie es completamente incapaz de autopolinizarse puesto que pierde sistemáticamente el polen de las anteras antes de la salida de los estilos. Como en otras malváceas, esta estrategia parece una adaptación para incrementar la polinización cruzada en detrimento del éxito reproductivo total. Esto puede beneficiar a la planta con mayores niveles de diversidad genética, pero puede comprometer su éxito reproductivo, al depender de unos visitantes florales que pueden no ser polinizadores eficientes.

Capítulo 4

El Elemento Ornitófilo Macaronésico se ha propuesto en base a las observaciones de visitas de aves a las flores para alimentarse de néctar y a los rasgos morfológicos observados en ellas. Es necesario examinar el grado de eficiencia como polinizadores de los paseriformes que actualmente mantendrían la interacción mutualista para comprobar las hipótesis sobre su origen y su persistencia. El rol de las aves generalistas se ha comprobado en algunas especies como *Lotus berthelotii* o *Canarina canariensis*. En *Navaea*, la hipótesis propuesta es la de un síndrome relicto originado en el continente en respuesta a aves nectarívoras especialistas. En este capítulo se estudian las características de la eficiencia de los polinizadores en *Navaea*, tanto desde el punto de vista cuantitativo (intensidad de las visitas a las flores) como cualitativo (retirada y deposición del polen, éxito de fructificación).

Se encontró una frecuencia elevada de visitas ilegítimas de abeja melífera y abejorro canario. En relación a las aves, las currucas cabecinegra y capirotada son polinizadores poco frecuentes pero más eficientes. Por otro lado el herrerillo canario y el mosquitero presentan patrones de frecuencia de visitas similares, pero con una marcada diferencia cualitativa, ya que solamente los mosquiteros y en menor medida las currucas permiten una fructificación efectiva. Las diferencias en la eficiencia como polinizadores pueden interpretarse en base al diferente comportamiento de las aves en las visitas a las flores. La polinización más eficiente se produce con las visitas con las aves posadas, tal y como hacen las currucas, pero también los nectarínidos africanos.

Capítulo 5

Además de la cuantificación de la presión de selección que los visitantes florales ejercen sobre los rasgos florales, es necesario explorar el papel de estos para interpretar la evolución del síndrome ornitófilo. Se dispone de escasos

datos sobre los caracteres florales en la mayoría de las especies del Elemento Ornitófilo Macaronésico. En este capítulo se analizan tres rasgos fundamentales de la biología floral: la capacidad de atracción de los polinizadores a través del estímulo visual y la recompensa del néctar, y la facilitación de la visita, según el tipo de células epiteliales presentes en los pétalos.

A pesar del color rojo asalmonado conspicuo de los pétalos, se detectó un pico de reflectancia ultravioleta, que convierte en perfectamente visibles para los insectos flores que de otro modo serían crípticas, lo que explica la alta frecuencia de visitas; por otro lado, se demuestra la masiva secreción de néctar diluido, y la ausencia de células estriadas en los pétalos que favorezcan la entrada de los insectos. Estos resultados sugieren que la estrategia reproductiva de *Navaea phoenicea* es resultado de una adaptación que maximiza la atracción de aves, sin excluir las visitas ilegítimas. Dado que la cantidad de recompensa es tan alta, el robo de néctar por parte de insectos no merma la capacidad reproductiva de la especie.

Capítulo 6

Este capítulo se centra en los factores de riesgo de la especie de estudio. Para ello se realizó el análisis de los censos anuales de efectivos llevados a cabo durante 10 años para averiguar tanto el estado actual de las poblaciones como las tendencias. Los censos sirvieron para determinar cuatro estadios de edad en los individuos, que se usaron para construir matrices de transición y determinar las tasas finitas de crecimiento y la tendencia determinística poblacional. Se contrastó el crecimiento finito anual con los valores climáticos mensuales. También se usaron los datos de crecimiento de las poblaciones para construir un modelo estocástico y determinar la probabilidad de extinción de las poblaciones. Por último, se comprobó de manera preliminar el efecto en el reclutamiento de la exclusión de herbívoros introducidos mediante un vallado.

Se encontró una prevalencia muy clara de individuos maduros, de mayor potencial reproductivo, mientras los individuos jóvenes eran más escasos. El número total de individuos censados fue significativamente inferior al censo previo disponible. La tendencia poblacional calculada resultó ligeramente decreciente en ambas poblaciones, con una tasa de crecimiento finito correlacionada fuertemente con la precipitación total a lo largo del año. La probabilidad de extinción calculada para el modelo estocástico fue coherente con los resultados del determinista, prediciendo una alta probabilidad de extinción en menos de 200 años. El experimento de exclusión reveló diferencias en el reclutamiento, pero con escaso poder estadístico. La estrategia de conservación de la especie se recomienda por tanto la preservación de los individuos reproductivos y las interacciones mutualistas con los polinizadores. La relación del clima con el crecimiento de la población obliga al control de las variaciones en el clima para asegurar la supervivencia de la especie.

REFERENCIAS

Ablay G.J. & Hürlimann M. (2000) Evolution of the north flank of Tenerife by recurrent giant landslides. *Journal of Volcanology and Geothermal Research*, **103**, 135–159.

Ancochea E. (2004) Canarias y el vulcanismo neógeno peninsular. *Geología de España* (ed. by J.A. Vera), pp. 635–680. SGE-IGME, Madrid.

Anguita F. & Hernán F. (2000) The Canary Islands origin: a unifying model. *Journal of Volcanology and Geothermal Research*, **103**, 1–26.

del Arco Aguilar M.-J. (2006) *Mapa de vegetación de Canarias.* La Laguna, Tenerife.

del Arco Aguilar M.J., González-González R., Garzón-Machado V., & Pizarro-Hernández B. (2010) Actual and potential natural vegetation on the Canary Islands and its conservation status. *Biodiversity and Conservation*, **19**, 3089–3140.

Báez M. (1992) Zoogeography and evolution of the avifauna of the Canary Islands. *Papers in avian paleontology* (ed. by K.E. Campbell), pp. 425–431. Natural History Museum of los Angeles County, Los Angeles.

Baker H.G. (1955) Self-compatibility and establishment after'long-distance'dispersal. *Evolution*, **9**, 347–349.

Bañares-Baudet Á., Blanca G., Güemes J., Moreno-Saiz J.C., & Ortiz S. (2004) *Atlas y Libro Rojo de la Flora Vascular Amenazada de España.* Dirección General de Conservacón de la Naturaleza, Madrid.

Bates D.M. (1968) Generic relationships in the Malvaceae, tribe Malveae. *Gentes Herbarum*, **10**, 117–135.

Bates D.M. & Blanchard O.J. (1970) Chromosome Numbers in the Malvales. II. New or Otherwise Noteworthy Counts Relevant to Classification in the Malvaceae, Tribe Malveae. *American Journal of Botany*, **57**, 927–934.

Borgen L. (1979) Karyology of the Canarian flora. *Plants and Islands* (ed. by D. Bramwell), pp. 329–346. London.

Boulesteix T., Hildenbrand A., Gillot P.-Y., & Soler V. (2012)

Eruptive response of oceanic islands to giant landslides: New insights from the geomorphologic evolution of the Teide–Pico Viejo volcanic complex (Tenerife, Canary). *Geomorphology*, **138**, 61–73.

Boulesteix T., Hildenbrand A., Soler V., Quidelleur X., & Gillot P.-Y. (2013) Coeval giant landslides in the Canary Islands: Implications for global, regional and local triggers of giant flank collapses on oceanic volcanoes. *Journal of Volcanology and Geothermal Research*, **257**, 90–98.

Brown R.P., Hoskisson P. a, Welton J.-H., & Báez M. (2006) Geological history and within-island diversity: A debris avalanche and the Tenerife lizard Gallotia galloti. *Molecular Ecology*, **15**, 3631–3640.

Carine M.A., Humphries C.J., Guma I.R., Reyes-Betancort J.A., & Santos Guerra A. (2009) Areas and algorithms: Evaluating numerical approaches for the delimitation of areas of endemism in the Canary Islands archipelago. *Journal of Biogeography*, **36**, 593–611.

Carlquist S. (1966) The biota of long-distance dispersal I. Principles of dispersal and evolution. *The Quarterly review of biology*, **41**, 247–270.

Castro-Urgal R. & Traveset A. (2014) Differences in flower visitation networks between an oceanic and a continental island. *Botanical Journal of the Linnean Society*, **174**, 478–488.

Caujapé-Castells J. (2011) Jesters, red queens, boomerangs and surfers: a molecular outlook on the diversity of the Canarian endemic flora. *The biology of islands floras* pp. 284–324. Cambridge University Press, Cambridge.

Cronk Q. (1992) Relict floras of Atlantic islands: patterns assessed. *Biological Journal of the Linnean Society*, **46**, 91–103.

Dupont Y.L., Hansen D.M., Rasmussen J.T., & Olesen J.M. (2004) Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. *Functional Ecology*, **18**, 670–676.

Emerson B.C. & Kolm N. (2005) Species diversity can drive speciation. *Nature*, **434**, 1015–1017.

Escobar García P., Schönswetter P., Fuertes Aguilar J., Nieto Feliner G., & Schneeweiss G.M. (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the Malva alliance (Malvaceae). *Molecular Phylogenetics and Evolution*, **50**, 226–39.

Faegri K. & Van Der Pijl L. (1979) *The principles of pollination ecology*. Elsevier, Oxford.

Fernández-Palacios J.M. & Nascimento L. De (2011) Political erosion dismantles the conservation network existing in the Canary Islands. *Frontiers of Biogeography*, **3**, 99–103.

Fernández-Palacios J.M., De Nascimento L., Otto R., Delgado J.D., García-del-Rey E., Arévalo J.R., & Whittaker R.J. (2011) A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography*, **38**, 226–246.

Fernández-Palacios J.M. & Whittaker R.J. (2008) The Canaries: an important biogeographical meeting place. *Journal of Biogeography*, **35**, 379–387.

García-Verdugo C., Sajeva M., La Mantia T., Harrouni C., Msanda F., & Caujapé-Castells J. (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. *Molecular Ecology*, **24**, 726–741.

Gobierno de Canarias (2010) Catálogo Canario de especies protegidas. **167**, 16.

Gómez-Campo C. (1996) *Libro rojo de especies vegetales amenazadas de las Islas Canarias*. Viceconsejería de Medio Ambiente, Gobierno de Canarias, Santa Cruz de Tenerife.

González-Castro A., Traveset A., Nogales M., Hansen D.M., Piazzon M., Larrinaga A.R., Rodríguez-Pérez J., Latorre L., Navarro L., & Santamaría L. (2012) Seed dispersal interactions in the Mediterranean Region: contrasting patterns between islands and mainland. *Journal of Biogeography*, **39**, 1938–1947.

Harter D.E.V., Irl S.D.H., Seo B., Steinbauer M.J., Gillespie R.G., Triantis K. a., Beierkuhnlein C., & Fernández-Palacios J.M. (2015) Impacts of global climate change on the floras of oceanic islands – Projections, implications and current knowledge. *Perspectives in Plant Ecology, Evolution and Systematics*, **17**, 160–183.

Heleno R.H. & Vargas P. (2015) How do islands become green? *Global Ecology and Biogeography*, **24**, 518–526.

Hürlimann M., Marti J., & Ledesma A. (2004) Morphological and geological aspects related to large slope failures on oceanic islands - The huge La Orotava landslides on Tenerife, Canary Islands. *Geomorphology*, **62**, 143–158.

Juan C., Emerson B.C., Oromí P., & Hewitt G.M. (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution*, **15**, 104–109.

Kaiser-Bunbury C.N., Traveset A., & Hansen D.M. (2010) Conservation and restoration of plant-animal mutualisms on oceanic islands. *Perspectives in plant ecology, evolution and*

systematics, **12**, 131–143.

Lens F., Davin N., Smets Erik, & Arco M. del (2013) Insular Woodiness on the Canary Islands: A Remarkable Case of Convergent Evolution. *International Journal of Plant Sciences*, **174**, 992–1013.

Lloyd D.G. (1992) Self- and Cross-Fertilization in Plants. II. The Selection of Self- Fertilization. *International Journal of Plant Sciences*, **153**, 370.

Machado A. (2002) La biodiversidad de las Islas Canarias. *La Diversidad Biológica de España* (ed. by F.D. Pineda, J.M. De Miguel, M.A. Casado, and J. Montalvo), pp. 89–99. Pearson Education, S. A., Madrid.

Mairal M., Sanmartín I., Aldasoro J.J., Culshaw V., Manolopoulou I., & Alarcón M.L. (2015) Paleo-islands as refugia and sources of genetic diversity within volcanic archipelagos: The case of the widespread endemic Canarina canariensis (Campanulaceae). *Molecular Ecology*, **24**, 3944–3963.

McArthur R.A. & Wilson E.O. (1967) *The theory of island biogeography*. Princeton University Press, Princeton.

Médail F. & Quézel P. (1999) Biodiversity Hotspots in the Mediterranean Basin: Setting Global Conservation Priorities. *Conservation Biology*, **13**, 1510–1513.

Moreno-Saiz J.C. (2008) *Lista Roja de Flora Vascular Española.* Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid.

Moreno-Saiz J.C., Domínguez Lozano F., Marrero-Gómez M. V., & Bañares-Baudet Á. (2015) Application of the Red List Index for conservation assessment of Spanish vascular plants. *Conservation Biology*, **29**, 910–919.

Moya O., Contreras-Díaz H.G., Oromí P., & Juan C. (2004) Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). *Molecular Ecology*, **13**, 3153–3167.

De Nascimento L., Willis K.J., Fernández-Palacios J.M., Criado C., & Whittaker R.J. (2009) The long-term ecology of the lost forests of la Laguna, Tenerife (Canary Islands). *Journal of Biogeography*, **36**, 499–514.

Nogales M., Heleno R., Rumeu B., González-Castro A., Traveset A., Vargas P., & Olesen J.M. (2015) Seed-dispersal networks on the Canaries and the Galápagos archipelagos: interaction modules as biogeographical entities. *Global Ecology and Biogeography*, **24**, .

Nogales M., Heleno R.H., Traveset A., & Vargas P. (2012) Evidence for overlooked mechanisms of long-distance seed dispersal to and between oceanic islands. *New Phytologist*, **194**, 313–317.

Ojeda D.I. (2013) The Macaronesian bird-flowered element as a model system to study the evolution of ornithophilous floral traits. *Vieraea*, **41**, 73–89.

Ojeda D.I., Santos-Guerra A., Oliva-Tejera F., Valido A., Xue X.., Marrero-Rodríguez Á., Caujapé-Castells J., & Cronk Q. (2013) Bird-pollinated Macaronesian Lotus (Leguminosae) evolved within a group of entomophilous ancestors with post-anthesis flower color change. *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 193–204.

Olesen J.M. (1985) The Macaronesian bird-flower element and its relation to bird and bee oppurtunists. *Journal of the Linnean Society*, **91**, 395–414.

Olesen J.M. (1988) Floral biology of the Canarian Echium wildpretii: bird-flower or a water resource to desert bees? *Acta Botanica Neerl*, 509–513.

Olesen J.M., Alarcón M.L., Ehlers B.K.., Aldasoro J.J., & Roquet C. (2012) Pollination, biogeography and phylogeny of oceanic island bellflowers (Campanulaceae). *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 169–182.

Ollerton J., Alarcón R., Waser N.M., Price M. V., Watts S., Cranmer L., Hingston A., Peter C.I., & Rotenberry J. (2009) A global test of the pollination syndrome hypothesis. *Annals of Botany*, **103**, 1471–80.

Reyes-Betancort J.A., Santos Guerra A., Guma I.R., Humphries C.J., & Carine M. a. (2008) Diversity, rarity and the evolution and conservation of the Canary Islands endemic flora. *Anales del Jardín Botánico de Madrid*, **65**, 25–45.

Rodríguez Núñez S., Acevedo Rodríguez A., Oval de la Rosa J.P., Matos J., Rodríguez Martín B., & Rodríguez Martín A. (2004) Lavatera phoenicea Vent. *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (ed. by Á. Bañares-Baudet, G. Blanca, J. Güemes, J.C. Moreno, and S. Ortiz), pp. 895. Dirección General de Conservación de la Naturaleza, Madrid.

Rodríguez-Rodríguez M.C., Jordano P., & Valido A. (2013) Quantity and quality components of effectiveness in insular pollinator assemblages. *Oecologia*, **173**, 179–90.

Rodríguez-Rodríguez M.C. & Valido A. (2008) Opportunistic nectar-feeding birds are effective pollinators of bird-flowers from Canary Islands: Experimental evidence from Isoplexis canariensis (Scrophulariaceae). *American Journal of Botany*, **95**, 1408–1415.

Rodríguez-Rodríguez M.C. & Valido A. (2011) Consequences of plant-pollinator and floral-herbivore

interactions on the reproductive success of the Canary Islands endemic Canarina canariensis (Campanulaceae). *American Journal of Botany*, **98**, 1465–1474.

Sanmartín I., Anderson C.L., Alarcón M.L., Ronquist F., & Aldasoro J.J. (2010) Bayesian island biogeography in a continental setting: the Rand Flora case. *Biology Letters*, **6**, 703–707.

Sanmartín I., Van Der Mark P., & Ronquist F. (2008) Inferring dispersal: A Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. *Journal of Biogeography*, **35**, 428–449.

Stebbins G.L. (1957) Self Fertilization and Population Variability in the Higher Plants. *The American Naturalist*, **91**, 337.

Stuessy T.F., Jakubowsky G., Gómez R.S., Pfosser M., Schlüter P.M., Fer T., Sun B.Y., & Kato H. (2006) Anagenetic evolution in island plants. *Journal of Biogeography*, **33**, 1259–1265.

Stuessy T.F., Takayama K., López-Sepúlveda P., & Crawford D.J. (2013) Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society*, **174**, 276–288.

Tate J.A., Fuertes Aguilar J., Wagstaff S.J., La Duke J.C., & Bodo Slotta T.A. (2005) Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoidae) as inferred from ITS sequence data. *American Journal of Botany*, **92**, 584–602.

Traveset A., Tur C., Trøjelsgaard K., Heleno R.H., Castro-Urgal R., & Olesen J.M. (2015) Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*, **24**, 1–11.

Trøjelsgaard K., Báez M., Espadaler X., Nogales M., Oromí P., Roche F. La, & Olesen J.M. (2013) Island biogeography of mutualistic interaction networks. *Journal of Biogeography*, **40**, 2020–2031.

Trøjelsgaard K. & Olesen J.M. (2013) Macroecology of pollination networks. *Global Ecology and Biogeography*, **22**, 149–162.

Valido A., Dupont Y.L., & Olesen J.M. (2004) Bird-flower interactions in the Macaronesian islands. *Journal of Biogeography*, **31**, 1945–1953.

Vargas P. (2007) Are Macaronesian islands refugia of relict plant lineages ? A molecular survey. *Phylogeography of Southern European refugia* pp. 297–314. Springer, The Netherlands.

Vargas P., Arjona Y., Nogales M., & Ruben H. (2015) Long-distance dispersal to oceanic islands : success of plants with multiple diaspore specializations. *Annals of Botany Plants*, 1–9.

Ventenat E.P. (1803) *Le jardin de la Malmaison vol. 2.* Imprimerie de Crapelet, Paris.

Vogel S., Westerkamp C., Thiel B., & Gessner K. (1984) Ornitophilie auf den Canarischen Inseln. *Plant Systematics and Evolution*, **146**, 225–248.

VVAA (2000) Lista Roja de la Flora Vascular Española. *Conservación Vegetal*, **6**, 1–44.

Warren B.H., Simberloff D., Ricklefs R.E., Aguilée R., Condamine F.L., Gravel D., Morlon H., Mouquet N., Rosindell J., Casquet J., Conti E., Cornuault J., Fernández-Palacios J.M., Hengl T., Norder S.J., Rijsdijk K.F., Sanmartín I., Strasberg D., Triantis K.A., Valente L.M., Whittaker R.J., Gillespie R.G., Emerson B.C., & Thébaud C. (2015) Islands as model systems in ecology and evolution: prospects fifty years after MacArthur-Wilson. *Ecology Letters*, **18**, 200–217.

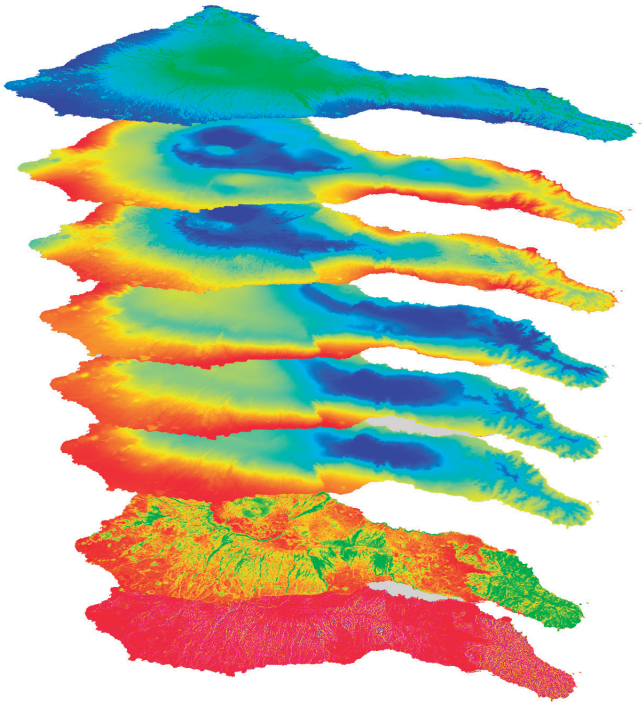
Webb P.B., Berthelot S., Goode G.B., Oudart P.L., & Valenciennes M. (1840) *Histoire naturell des Iles Canaries vol.2 (1)*. Bethune, Paris.

Whittaker R.J. & Fernández-Palacios J.M. (2007) *Island biogeography: ecology, evolution, and conservation.* Oxford University Press, Chippenham.

Whittaker R.J., Ladle R.J., Araùjo M.B., Fernández-Palacios J.M., Delgado J.D., & Arévalo J.R. (2007) The island immaturity - speciation pulse model of island evolution: an alternative to the "diversity begets diversity" model. *Ecography*, **30**, 321–327.

1 The use of species distribution modelling for discerning recent fragmentation from ancient disjunction in volcanic islands

El uso de modelos de distribución de especies para diferenciar la fragmentación reciente de la disyunción antigua en islas volcánicas



The use of species distribution modelling for discerning recent fragmentation from ancient disjunction in volcanic islands

ABSTRACT

Disjunct distribution areas on volcanic islands are the result of historical, ecological and/or fragmentation processes. We performed a high-resolution niche modelling study to identify which environmental factors related to topography, climate and human disturbance best explain the disjunct distribution of a palaeoendemic species. *Navaea phoenicea* is distributed only on the northern slopes of the two oldest volcanic palaeoislands (Teno and Anaga massifs of Tenerife), which are separated by a 70-km-wide gap. An exhaustive sampling of N. phoenicea locations was conducted. Three high-resolution sets of bioclimatic, topographic and spatial predictor maps were developed. We then used three approaches to assess the contributions of these different factors to the distribution of N. phoenicea: species distribution models (SDMs; four algorithms: generalised linear models, general additive models, generalised boosting regression and random forest), hierarchical partitioning and variance partitioning. The four algorithms provided congruent predictions of a potential distribution primarily restricted to palaeoislands, with limited exceptions on steep slopes outside of the actual distribution. The scattered suitable areas connecting the Teno and Anaga core areas are situated in areas subject to recent catastrophic events or human disturbance. The main ecological factors as inferred from the hierarchical partitioning and variance partitioning analyses revealed the greater predictive power of topographic factors (slope) relative to climate constraints such as evapotranspiration. Our study shows that the disjunct distribution of N. phoenicea is best explained by the current topography, which is highly shaped by geological processes on this island. This opens the question whether the species is primarily adapted to these environments or restricted to them due to exclusion from other habitats by biotic (competition) or negative anthopogenic influence. Further studies should use complementary ecophysiological and experimental (e.g. transplant) approaches to disentangle the ultimate cause of this disjunction. Our results also suggest the existence of suitable areas in-between disjunct populations, where one should verify whether relict populations have persisted until recent times.

INTRODUCTION

Development of novel approaches for the study of causes of spatial patterns can provide new insights in the understanding of dynamics and conservation of populations in oceanic islands. From Darwinian times, we accept that the evolutionary fate of island lineages is influenced by factors such as successive stages of volcanism, eustatic sea-level changes (Carine *et al.*, 2004), dissimilar relative colonisation ages, unequal evolutionary rates (Price & Clague, 2002), novel ecological interactions, and ecosystem disruption by human activities which often overlap along time to result in complex spatial patterns. Volcanic islands are among the most threatened ecosystems in the world (Millennium Ecosystem Assessment, 2005). Due to their small area, severe fragmentation and habitat destruction caused by anthropic activity together with small population sizes, are the most critical threats on insular species (Kier *et al.*, 2009; Caujapé-CWastells *et al.*, 2010). Therefore, at such limited geographic scales, the correct interpretation of causes of spatially fragmented patterns becomes critical for conservation initiatives (Whittaker *et al.*, 2005).

Disjunct species distributions and fragmented populations on islands have been recurrent topics in studies of bio- and phylogeographic patterns in plants and they have been addressed on large islands of cratonic origin, such as New Zealand (Heads & Craw, 2004; McDowall, 2008), New Caledonia (Heads, 2008), Madagascar (Perry *et al.*, 2013) or Cuba (Jestrow *et al.*, 2012). Although the use of species distribution modelling (SDM) to associate island biodiversity, ecoclimatic factors and habitat suitability (Hortal *et al.*, 2007; Vanderpoorten *et al.*, 2007) , no attempt is known, to our knowledge, of its application to untangle the putative causes for disjunct areas of endemic species in oceanic islands.

The use of SDM within volcanic islands may present additional methodological challenges related to their geological origin. In many instances, spatially explicit and ecologically-consistent, climatic and geological data are lacking. This is particularly important where, as a result of the ecological heterogeneity, it is required to conduct fine-scale studies at the dimension of volcanic islands or small habitats (e.g. Price, 2004). At such limited spatial scales, issues related to conservation management and fragmentation become critical aspects highlighted by conservation biogeography disciplines (Whittaker *et al.*, 2005)

The Canary Islands are one of the best-studied models in island biogeography. Research has focused on colonisation and radiation processes (Emerson & Oromí, 2005; Whittaker *et al.*, 2007; Caujapé-Castells, 2011) and the study of patterns of distribution among and within islands (e.g. Carine *et al.*, 2009), and also on conservation genetics of endangered species (Reyes-Betancort *et al.*, 2008; Caujapé-Castells *et al.*, 2010). An interesting case of disjunction is found on the island of Tenerife, where at least eleven endemic plant species have been reported to share a discontinuous distribution among isolated two of the three primitive areas of the island: the western (Teno) and eastern (Anaga) mountain massifs (Moya *et al.*, 2004; Trusty *et al.*, 2005). The pattern is also remarkable because both Anaga and Teno massifs constitute areas of endemism (Reyes-Betancort *et al.*, 2008; Carine *et al.*, 2009).

Navaea phoenicea (Vent.) Webb & Berthel. (Malvaceae) is a palaeoendemic arborescent mallow from Tenerife (Tate & Fuertes Aguilar, 2005; Escobar García *et al.*, 2009). The conservation status of the species, together with the evolutionary distinctiveness as part of the notable ornithophilous Canarian flora, raises a particular interest in this species. Its conservation status under UICN categories falls under EN or VU categories (Rodríguez Núñez *et al.*, 2004; Moreno-Saiz, 2008). Threats identified include seed dispersal limitations (Gómez-Campo, 1996), habitat degradation (Bañares-Baudet *et al.*, 2004) and genetic drift. Present populations inhabit cliffs ranging from 350 to 650 m a.s.l. between the thermophilous woodland and the laurel forest vegetation belts. These habitats are potentially distributed across the northern slopes of the island markedly determined by the incidence of trade winds at this altitudes (del Arco Aguilar *et al.*, 2010).

The species exhibits a disjunct distribution between the Teno and Anaga massifs, for which determining the causes of such a pattern requires proposing different possible scenarios compatible with such disjunction. The role of geological factors is reflected by the history of the island, as the current island geomorphology is the result of a succession of eruptive and destructive processes, encompassing volcanism and massive landslides (Solana & Aparicio, 1999; Llanes *et al.*, 2003; Walter *et al.*, 2005, figure 1). These events resulted in the union of the three palaeoislands into a single land mass, which was later re-

modelled by massive landslide episodes that have been especially relevant in configuring the current topography (Hürlimann *et al.*, 2004). Based both on geological history and the recent human colonization highlights, three alternative scenarios that encompass possible biogeographical explanations can be considered—two geology-based and one caused by human fragmentation—to explain the current disjunction in N. phoenicea on Tenerife.

The first two historical scenarios involve a geological basis supporting the disjunction. In the simplest scenario (S1), palaeoislands hosted separate populations before they were united into a single island. Under this scenario, the species would lack suitable habitats outside of its present distribution. In the second scenario (S2), N. phoenicea populations were once present throughout the northern belt and the current distribution is shaped by complex geological history (figure 1). Over time, landslides, which started to occur in the last 3 MY may have removed putative central populations, isolating the two relict populations of Teno and Anaga. For instance, during the Cañadas edifice III phase (1.4-0.17 MY), structural instabilities led to giant landslides, resulting in the opening of the current valleys of La Orotava and Icod de los Vinos in the north and Güimar in the South (Ablay & Hürlimann, 2000; Ancochea, 2004). Isolated populations may then have been prevented from recolonizing the central areas, again due to either a lack of suitable habitats or to dispersal limitations, as the species disperses only by barochory and rarely by endozoochory by lizards. Seed dispersal by lizards is frequent in the Canary Islands (Olesen & Valido, 2003) but for N. phoenicea it appears to be an exceptional mechanism. The same scenario has been postulated for plants (Mairal *et al.*, 2015), insects (Moya *et al.*, 2004) and lizards (Brown *et al.*, 2006). In the third scenario (S3), the distribution of *Navaea phoenicea* was once continuous but became fragmented as a result of human activities. Thermophilous and laurel forests had potentially occupied a continuous strip along the north façade of the island before human disturbance, although it is likely that vegetation composition has undergone a significant variation due to climate dynamics along the Holocene period (Nogué *et al.*, 2013). First the settlers arriving from the Canaries and later activities associated with European land reclamation (Carmen Machado Yanes *et al.*, 1997; Rando *et al.*, 1999; del Arco Aguilar *et al.*, 2010) strongly affected the extent and composition of these forests across the central areas of the island (De Nascimento *et al.*, 2009; Fernández-Palacios *et al.*, 2011). Some areas in the laurel forest in Teno and Anaga mountain massifs have remained better conserved than the central areas thanks to conservation policies. On the contrary, a great part of the thermophilous forest has been to agricultural land use as well, and they currently constitute recovery areas because farmlands on the steepest slopes have been abandoned. Under this scenario, the process leading to the present population distribution would be human-driven fragmentation rather than a palaeoecological disjunction.

Thus, two hypotheses (H1-H2) built upon scenarios S1-3 can be postulated for the potential distribution of the species. These hypotheses are not mutually exclusive, as the underlying factors can either overlap in time or encompass different temporal scales:

(H1) the potential distribution is restricted to palaeoislands, in topographic situations shaped by geological history; in areas not affected by landslides;

(H2) the species can potentially be distributed continuously throughout the northern slope of Tenerife, but the species was removed from the most accessible habitats under heavy human influence.

Species distribution model (SDM, Guisan & Thuiller, 2005) predictions can be used to explore the potential causes of the current disjunction of N. phoenicea as they provide partial insights into which environmental factors shape the species ecological niche suitability. In this paper, we assess these concurrent hypotheses through a combination of SDM, and two techniques that allow assessing the joint and individual contributions of spatial, topographic and bioclimatic factors and groupings of them: hierarchical partitioning (HP; Chevan & Sutherland, 1991) for individual variables and variance partitioning (VP; Borcard *et al.*, 1992) for groups of variables. In recent years, SDM have proven to be useful tools with which to assess biogeography and conservation issues (Guisan & Thuiller, 2005; Franklin, 2010; Peterson, 2011; Guisan *et al.*, 2013) including case studies in the Canary Islands (Hortal *et al.*, 2007; Aranda & Lobo, 2011; Irl *et al.*, 2015).

Although a formal disentanglement of factors affecting N. phoenicea distribution by hypothesis testing is not feasible with correlative SDM, these can point to some important factors and contribute partially to assess how much support each of the tow hypotheses obtain. For instance, if a continuous potential distribution is predicted by SDMs then a recent disjunction due to

habitat fragmentation is more likely. But if a disjoint distribution is predicted, one cannot know whether it results from the influence of geological history on potential distribution, or if the species was excluded from the other potential habitats by human influence or another factor (e.g. missing predictor, biotic interactions). Another example relates to the contribution of bioclimatic versus topographic factors. If climate results to be the main explanatory factor of the current distribution, it would be a good support that climate rather than other factors correlated with topography (e.g. human influence) is driving the distribution. Correlative SDMs thus provide useful information to assess how much support each – or several – hypotheses obtain, and therefore contribute to better understand the species ecology for conservation purposes, but such study cannot lead to any definitive conclusion about causation. This study also contributes partially to assess the extent of human disturbance on the species populations and allow indicating putative areas for its reintroduction.

METHODS

Sampling

All known populations of *Navaea phoenicea* were visited between 2005 and 2008, taking advantage of previous accurate records from the BIOTA database (http://www.biodiversidadcanarias.es/atlantis) and complementing these records with additional field records. The locations of individuals were recorded using a GPS Garmin eTrex Vista with a precision of 5 to 8 m. To minimise sampling error, coordinates delimiting the contours of patches of individuals and distances between them were also considered. Presence points were converted into a presence-only layer at a 20 m raster cell resolution, to get a final dataset of presences in an ArcGIS framework (ESRI, 2004). The remaining cells without presence were considered true absences in subsequent analyses, as we counted with precise knowledge of the population realized distribution. However, because our goals wanted to estimate the species' potential natural distribution, constraints other than relevant environmental factors were avoided. For this reason, we avoided selecting absences in human-altered habitats and created a random dataset of 10,000 absences within the remaining natural vegetation patches. To further minimise potential biases due to model

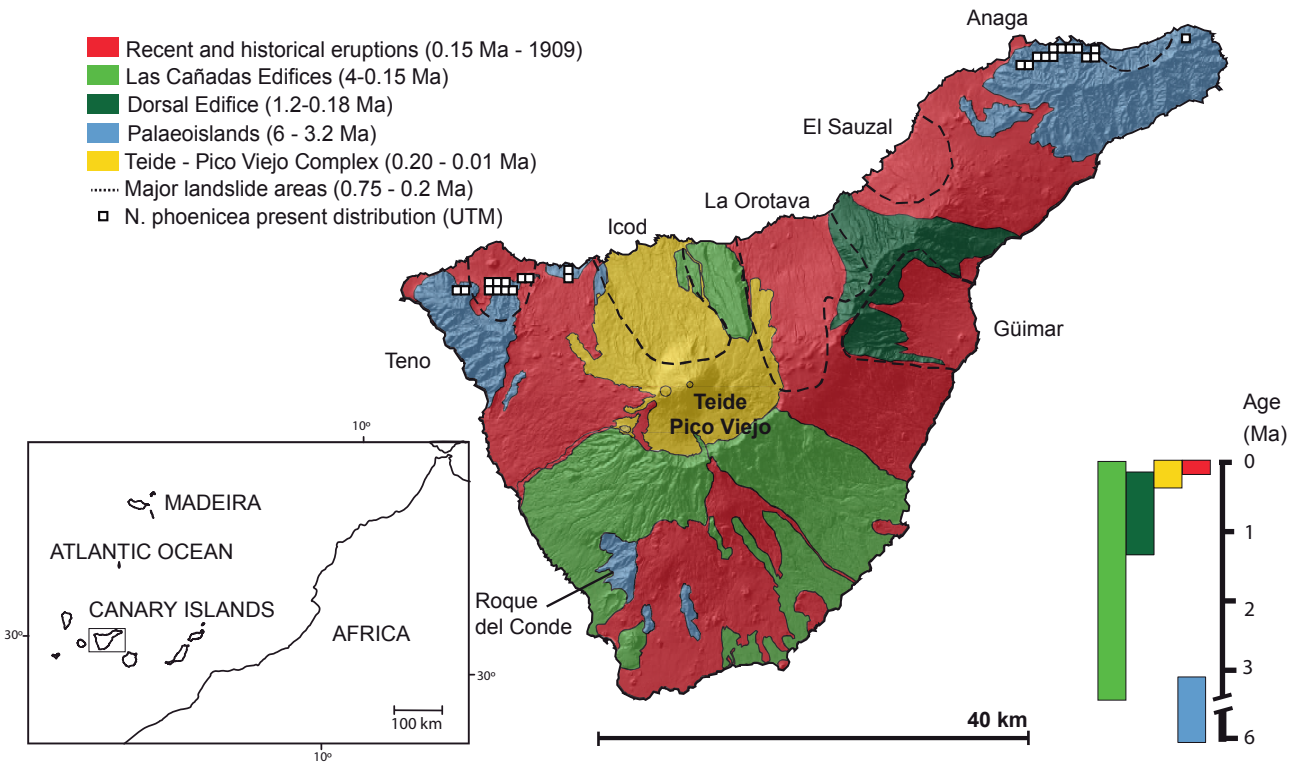


Figure 1. Map of Tenerife indicating the different volcanic stages and large-scale landslides. White squares represent 1-km-per-side squares within which *Navaea phoenicea* has been recorded.

design, absences were restricted to vegetation types in which *N. phoenicea* is actually found, following Del Arco Aguilar *et al.* (2006), including laurel and thermophilous forests and, less commonly, succulent coastal shrubland. Additionally, a 100 m buffer around presences was created where absences were not allowed.

Environmental predictors

We developed a set of environmental maps to be used as predictors in the SDM at a 20 m resolution. The selected variables are listed in table 1. For the calculation of topographic variables, we first extracted altitude data from the digital elevation model of the island. Slope and aspect were then derived using the Spatial Analyst extension in ArcGIS Desktop. Eastness and northness were defined as the sine and cosine of aspect, respectively (Lassueur *et al.*, 2006). Finally, topographic position was calculated with Arc Macro Language (AML) routines performed with the ArcInfo GRID module (AML scripts available on N. E. Zimmerman's website: http://www.wsl.ch/staff/niklaus.zimmermann/programs/aml.html). For climatic predictors, monthly data on precipitation and temperature from 275 climatic stations in the Tenerife agro-climatic network were used as response variables in a stepwise generalised additive model (GAM) using the following predictor variables: 1) for precipitation: altitude, northness, x and y coordinates; 2) for temperature: altitude, northness, slope, x and y coordinates. Model quality was assessed on the basis of the percentage of variance explained by the model. The residuals of the values of meteorological stations were mapped and interpolated by splines using the Spatial Analyst module in ArcGIS. We used fixed weighting considering only 4 points as nearest neighbours given the high density and distribution of data points. The resulting 12 maps for climate and temperature were used to calculate the seasonality of precipitation following Hijmans *et al.* (2005), mean aestival temperature and precipitation (June-September), as the species loses leaves during summer and the first rains at the end of this period appear to trigger the flowering period. Finally, we developed potential evapotranspiration (PET) using ArcInfo's built-in AML procedures. To this end, we first generated a theoretical solar radiation map using Zimmerman's corrected script of the original formula proposed by Kumar *et al.* (1997). The resulting monthly maps were combined with monthly temperature maps to generate a set of evapotranspiration maps. To assess the effect of spatial structure on the species presence, we also incorporated the number of adjacent cells with presence data as a spatial autocorrelation predictor for the analysis.

Species distribution modelling

We used BIOMOD package (Thuiller *et al.*, 2009) implemented in R software (R Core Team, 2013). We selected 4 algorithms: two traditionally used methods, namely, generalised linear models and generalised additive models (GLM and GAM; see Guisan *et al.*, 2002), and two novel tree-based machine-learning methods, namely, generalised boosted regression models (GMB, Elith *et al.*, 2008) and random forest (RF; Breiman, 2001). We performed 100 runs for each method, randomly selecting 111 absence points from the total pool of 10,000 in each run. 70% of the data was randomly selected for model calibration, and the remaining 30 % was used for model evaluation. Models were evaluated based on the area under the curve (AUC; Swets, 1988). We plotted an ecological suitability map (i.e., potential distribution) in ArcGIS from the mean suitability value of the 100 runs of each model.

We note here that our focus area is the northern area of the island. Nevertheless, models were also repeated using absence generation unconstrained for the whole island for eventual comparisons between results to avoid misleading

Table 1. Description of chosen predictors for species distribution models and hierarchical partitioning

Environmental group	Variable	Description
Topography	Slope	Maximum rate of change in height value from each map cell to its neighbors
Topography	Topography Index (TPI)	Topographic exposure at various spatial scales, hierarchically integrated into a single grid.
Climate	Annual Precipitation	Total precipitation
Climate	Aestival Precipitation	Total precipitation between June and September
Climate	Annual mean temperature	Mean annual temperature
Climate	Aestival temperature	Mean temperature between June and September
Climate	Precipitation seasonality	Coefficient of variation of precipitation through the year
Climate	Evapotranspiration (PET)	1/10 Mm of water evaporated
Spatial	Autocovariation	Number of adjacent cells with the species presence

interpretations when SDM results were extrapolated to the whole island.

This ecological modelling approach can show several methodological strengths related to the reliability of the modelling process (Araùjo & Guisan, 2006; Zimmermann *et al.*, 2010): 1) using the natural delimitation of an island avoids the subjectivity of defining a study extent; 2) we relied on an accurate sampling of species presences; 3) we benefited from a good initial knowledge of the ecological factors likely to control the species distribution; 4) we relied on reliable true absence data, avoiding most of the risks associated with their generation (Chefaoui & Lobo, 2008; Hengl *et al.*, 2009; VanDerWal *et al.*, 2009; Li *et al.*, 2011), and presence/absence approaches have been suggested to be more informative than presence-only methods (Elith *et al.*, 2009) (Elith & Graham, 2009); 5) we take advantage of fine-resolution spatial dataset that optimally accommodates the study extent and the scale at which relevant variations in the selected environmental predictors can be detected; and 6) by pre-selecting predictor variables based on prior ecological knowledge, we avoided using subjective model selection approaches and high-variance processes (e.g., stepwise selection for GLMs; see Guisan *et al.*, 2002).

Hierarchical partitioning

We used a hierarchical partitioning (HP) approach (Chevan & Sutherland, 1991) to assess the relative importance of individual predictors (Quinn & Keough, 2002; Heikkinen *et al.*, 2004). This procedure was conducted with the *hier.part* package (Mac Nally & Walsh, 2004) in R software using the entire set of variables. Because random forest and boosting regression trees response surfaces are not necessarily linear, we also accounted for variable importance outputs from models for a more straightforward interpretation.

Variance partitioning

Variance partitioning (VP) was conducted following the methodology proposed by Anderson and Gribble (Anderson *et al.*, 1998; see also Carrete *et al.*, 2007; Carrascal *et al.*, 2008; Diniz-Filho & Bini, 2008). Environmental predictors were grouped into two sets: climatic or topographic. We further considered a third set of predictors—spatial structure—consisting of the spatial autocovariate measure used previously together with the linear and quadratic terms of the geographical coordinates of the occurrence data. The fraction of explained variance for each of the three groups was obtained by performing a series of redundancy discriminant analyses (RDA) in which we combined each group as variable predictors alone or used the rest of the groups as co-variables. All analyses were performed with the VEGAN package (Oksanen *et al.*, 2010) in R software. For each RDA run, a forward selection of variables was performed with 9,999 Monte Carlo permutation tests. The total eigenvector inertia values obtained in the RDAs were taken as a measure with which to calculate single and joint fractions of variance from each group.

RESULTS

Species distribution modelling

The prospections of the populations yielded 111 ocurrences at the resolution of 20 meters. This resulted in an actual area of occupancy of only 0.044 km^2. Model evaluation proved high AUC (› 0.9) for all models (Swets, 1988). The mean AUC for GBM was significantly higher than that of the other models (figure 2).

The mean ecological suitability of the 100 runs exhibited very similar spatial patterns (figure 3), designating suitable areas primarily in the northern ravines of Teno and Anaga. The models identified few areas with high suitability values between palaeoislands where the species is currently distributed. These areas are 1) La Orotova Valley, 2) Güimar Valley, 3) the El Sauzal area and 4) the lowlands of the Dorsal Edifice between the former three.

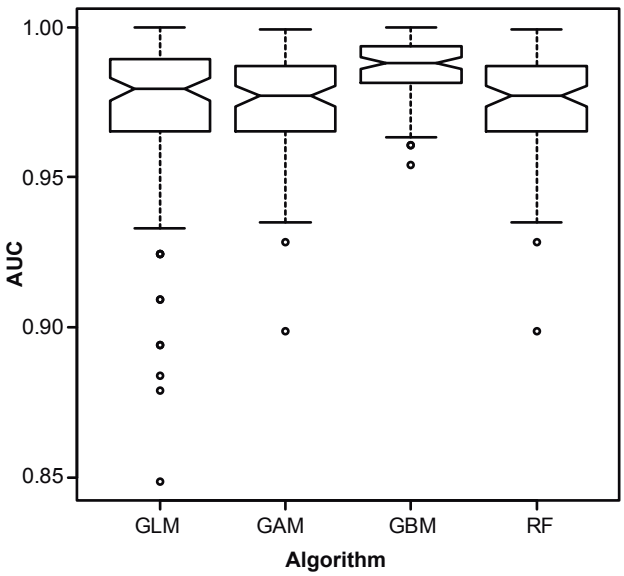


Figure 2. Boxplots of AUC scores for the 4 performed algorithms. GLM: generalised linear models; GAM: generalised additive models; GBM, boosting regression; RF: random forest.

Both valleys exhibit similar conditions of exposition and topography, including steep, shady slopes. These two areas have experienced landslides but not recent eruptions. Potentially suitable habitats identified in areas 3 and 4 include narrow, shady valleys with similar conditions.

However, differences were observed between models. GBM and RF predicted the largest potential areas indicating suitable zones in shaded areas in the south. In high-altitude areas of Las Cañadas del Teide National Park (2.500-3-718 m a.s.l.), suitability scored up to 0.40. GBM showed a more extended potential distribution than RF. Models developed with absences spanning across the whole island provided very similar results (data not shown).

Variance partitioning

Together, the selected variables explained a large proportion of the retained variance (84.1 %; figure 4). The largest fraction of variance was largely accounted for by the joint effect of the three groups—climatic, geological and spatial—(34.4 %) and could not be attributed to spatial, climatic or topographic groups on their own. Similar values were found for topographic variables alone and together with climatic variables (18.7 % and 17.9 %, respectively). Climatic variables alone explained the lowest percentage (2.2 %), which was even lower than that of their joint contribution with spatial variables. This latter group explained only 4.5 %. Thus, topography alone explained a higher proportion of variance, whereas the explanatory power of climate relied on other factors.

Hierarchical partitioning

The most important factors were slope and PET, accounting independently for 29.2 % and 27.11 % of variance, respectively (figure 5, table 2). The joint contribution of slope increased up to 27.6 %, whereas the explanatory power of PET decreased by a similar percentage (24.7 %). Spatial correlation was the third most significant variable, with 19.6 % of variance explained for independent explanatory power and a slight decrease for the joint contribution (17.6 %). The remaining climatic factors represented minor independent contributions, with the exception of mean temperature, although this factor represented only a minor joint contribution. Topographic position made an insignificant contribution. Hierarchical partitioning reflects the importance of slope for the species distribution. According to the differences between joint and independent variances, the climate contribution depends on other factors, such as altitude, slope and aspect. Variable importance calculations from the GBM and RF models, however, produced different results for temperature and precipitation variables: annual precipitation was markedly more important in RF (11.06); aestival precipitation and aestival temperature were near 0 in GBM (0.29 and 0.06), whereas PET scored 57.46. In HP, annual mean temperature was greater, and precipitation seasonality was lower.

DISCUSSION

Our results provide several insights into the causes of the disjunction of *N. phoenicea* in Tenerife. Model predictions identified suitable habitats as being mainly restricted to the Teno and Anaga massifs, with scarce areas in the centre of the island. Hierarchical partitioning and variance partitioning further pointed to topographical variables as the most important explanatory factors, but with a large variance shared with climate. Our results therefore do not support unambiguously any of the three hypotheses, as the causes of the predicted disjunction may equally be topo-climatic or due to a large exclusion by human from suitable areas in more gentle slopes in more densely human-occupied areas. Hereafter, we discuss our model and variance partitioning results and the various interpretations of the causes of disjunction in more details.

Interpretation of species ecology

The hierarchical and variance partitioning approaches showed the extent of the contributions of topographic and climatic factors to defining the species' ecological niche. However, the results from the two approaches provide different insights into variable contributions. In VP, most of the variance cannot be assigned to any of the three groups alone. Even where VP indicates that topography contributed more to the variance (12 %) than climate (2 %), because almost all the variance accounted for by climate is shared with topography, the largest contribution to variance cannot be assigned to either topography or climate alone. Conversely, HP provides clearer information regarding the factors contributing to variance in addition to insights into the shared contribution between environmental groups, whereas the comparison between the contribution of factors using RF and GBM is not consistent across all climatic variables. Specifically, temperature predictors were much less important for the

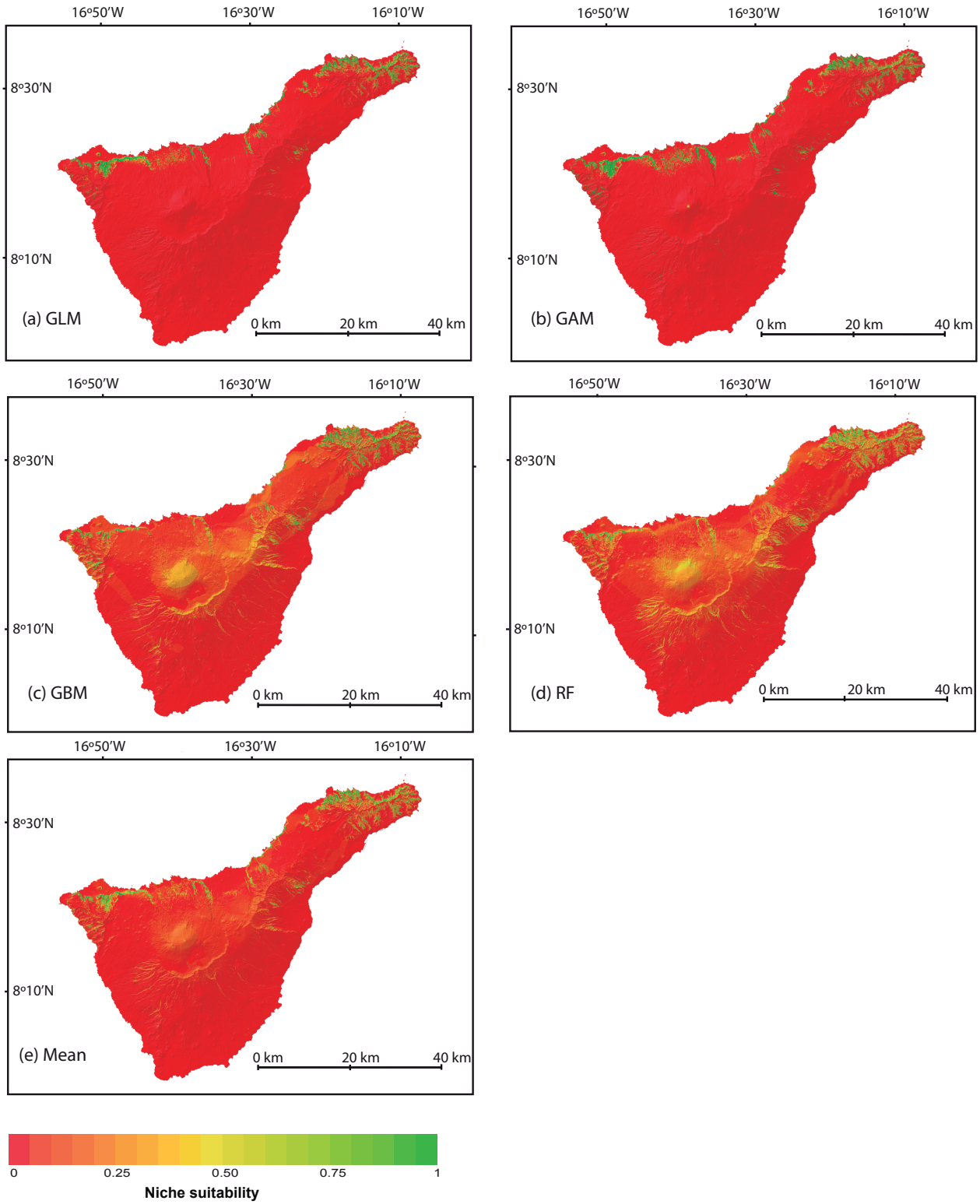


Figure 3. Maps of average environmental suitability calculated from 100 iterations of each algorithm employed and the averaged map of the four.

latter models. This fact can explain the broader habitat suitability among high-altitude (and low-temperature) areas predicted by these models.

The explanatory power of topographic variables relies on slope rather than on topographic position, which can be interpreted on the basis of the strong reliance of seed dispersal in barochory. Indeed, the age structure in *N. phoenicea* populations is correlated with altitude in each ravine, with older individuals at the highest elevations and younger individuals at lower elevations. A steep slope favours then seed dispersal. Besides the outcome of the models, a common situation is that these zones

are better conserved because of their inaccessibility for agriculture and introduced grazing animals (Irl *et al.*, 2014). However several populations grow in terraced crop fields, with several dated individuals older than the time of abandonment of the fields, whereas no individuals were recorded in well conserved areas with gentle slopes. An additional consideration is the role of topography at the local scales at which SDM were applied, which was shown to be relevant to the distribution of *N. phoenicea*. There is growing evidence in the literature emphasising the interplay of the spatial resolution of SDM and the importance of other environmental factors (Franklin *et al.*, 2013). Specifically, topography is relevant for local refugia (Austin & Van Niel, 2011). Therefore, other factors related to terrain properties, such as soil composition, drainage or organic matter that accumulates in microhabitats in ravines remain to be explored at a finer scale (Pradervand *et al.*, 2013). In the neighbour island of La Palma, Irl *et al.* (2015), found a differentiated role for climate and topography on biogeographic and speciation processes, indicating that environmental constraints operate at two different ecological levels.

Furthermore, the variance explained by climate partly indicates the role of the selected climatic factors, especially PET. This variable has a prominent role pertaining to humidity requirements and explains the prediction of suitable habitats in shady ravines, even in the south, where a moist environment is maintained. However, PET strongly depends on the topographic properties that determine the degree of direct solar radiation received. This fact explains the amount of variance shared between climatic and topographic groups in the VP analysis. In HP, this effect of slope on PET explains the decrease in the joint variance between climate and topography. Factors based on temperature and precipitation (aestival, annual and seasonality) are also influenced to some extent by topography, although factors such as altitude that directly affect the variables were not considered in order to minimise the correlation between variables. Concerning temperature, an evident altitudinal gradient exists that limits the distribution of the species to the lowlands. However, the overlap of the explanatory joint power of this variable with other factors is lower because of the presence of the more relevant factors noted above. Regarding precipitation, the low scores of precipitation factors can be explained based on the complex pattern of rainfall on the island and the fact that *N. phoenicea* populations are present in a broad spectrum of habitats ranging from coastal desert vegetation to laurel forest, covering almost the whole range of annual precipitation values recorded for the island.

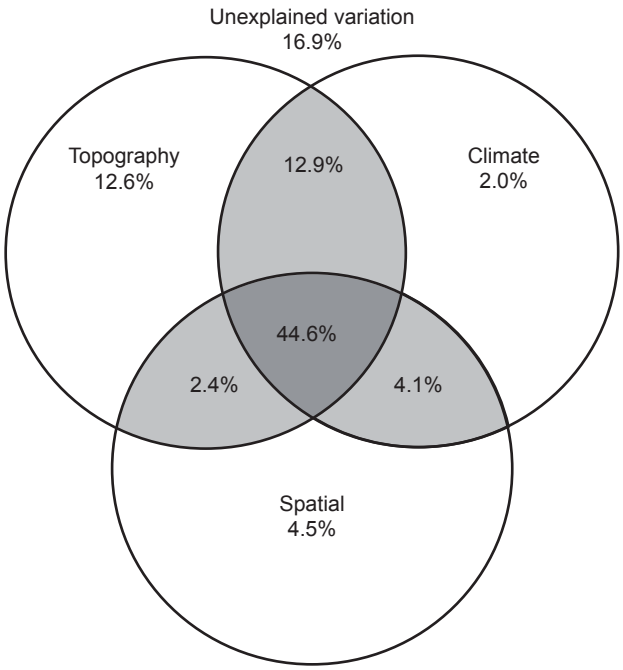


Figure 4. Diagram showing the percentages of variance explained for each group of predictors (see Table 1) obtained from the variance partitioning analysis. Percentages of shared variance between groups and unexplained variance are also shown.

These results indicate the importance of geological history relative to climatic factors. However, this conclusion can only be asserted based on the independent contributions of factors. In fact, the strong spatial aggregation of individuals accounts for an important proportion of the variance explained, which limits the contribution of these results to understanding the species distribution.

Table 2. Contributions of environmental predictors for Hierachical Partitioning (HP, independent contributions), Generalized Boosted Models (GBM) and Random Forest (RF) approaches. Data is given as the percentage of total explained variance.

Algorithm	Slope	TPI	Annual precipitation	Aestival precipitation	Annual Mean temperature	Aestival temperature	Precipitation seasonality	PET	Spatial correlation
HP	29.02	1.04	4.21	4.43	13.80	8.30	3.92	27.11	14.23
GBM	20.90	1.10	2.66	0.29	2.86	0.06	14.60	57.46	11.19
RF	16.65	4.02	11.06	5.56	4.78	2.01	11.69	20.59	9.09

Implications of SDM in biogeography

SDM results support mainly hypothesis H1, which formulates an intermediate scenario consisting of a distribution primarily restricted to Teno and Anaga plus additional intermediate areas in the northern slopes potentially suitable according to environmental factors, in which any possible existing population would have been extinguished recently. The possible detection of high pollen flow between Teno and Anaga detected with molecular markers would support the existence of these intermediate "bridge" populations (chapter 2). Therefore the species probably occupied already a restricted habitat at least at the moment of human occupation. SDM can help to designate new potential areas for a putative population assisted colonization. The identification of suitable areas between Teno and Anaga can be especially useful for genetic connectivity both populations.

Predicted potential areas were congruent among different algorithms and reflected previous prospective knowledge of the species' ecology, where suitable areas are found primarily on steep slopes. These habitats are present in the north, from the laurel forest to the edge of the coastal desert spurges formations, but are also found marginally in the south in shady ravines that compensate for lower precipitation and can host semi-permanent water flows.

Isolated topo-climatic suitable areas also exist outside of the current distribution of the species, particularly on slopes in central areas of the island flanking the valleys of Güimar and La Orotava as well as El Sauzal (figure 3). The first two valleys have origins in young landslide events, 730-560 ky for La Orotava (Ablay & Hürlimann, 2000) and 840 ka for Güimar (Seisdedos *et al.*, 2012), and can be potential areas for recolonisation. The remaining natural vegetation patches in these areas are extremely reduced, making it difficult to assess whether they were once present and later destroyed by human activity. The third suitable area, El Sauzal, has been affected by large landslides (Ablay & Hürlimann, 2000), the formation of the Dorsal Edifice and historical eruptions (Ancochea, 2004, see figure 1). Part of these suitable zones has subsequently been destroyed by water erosion. The suitability of these areas may also be constrained because of limited soil development.

Our results indicate that suitable areas for *N. phoenicea* are mostly restricted to the oldest cores of the island of Tenerife, constrained especially by slope. The current topography does not create suitable climatic conditions

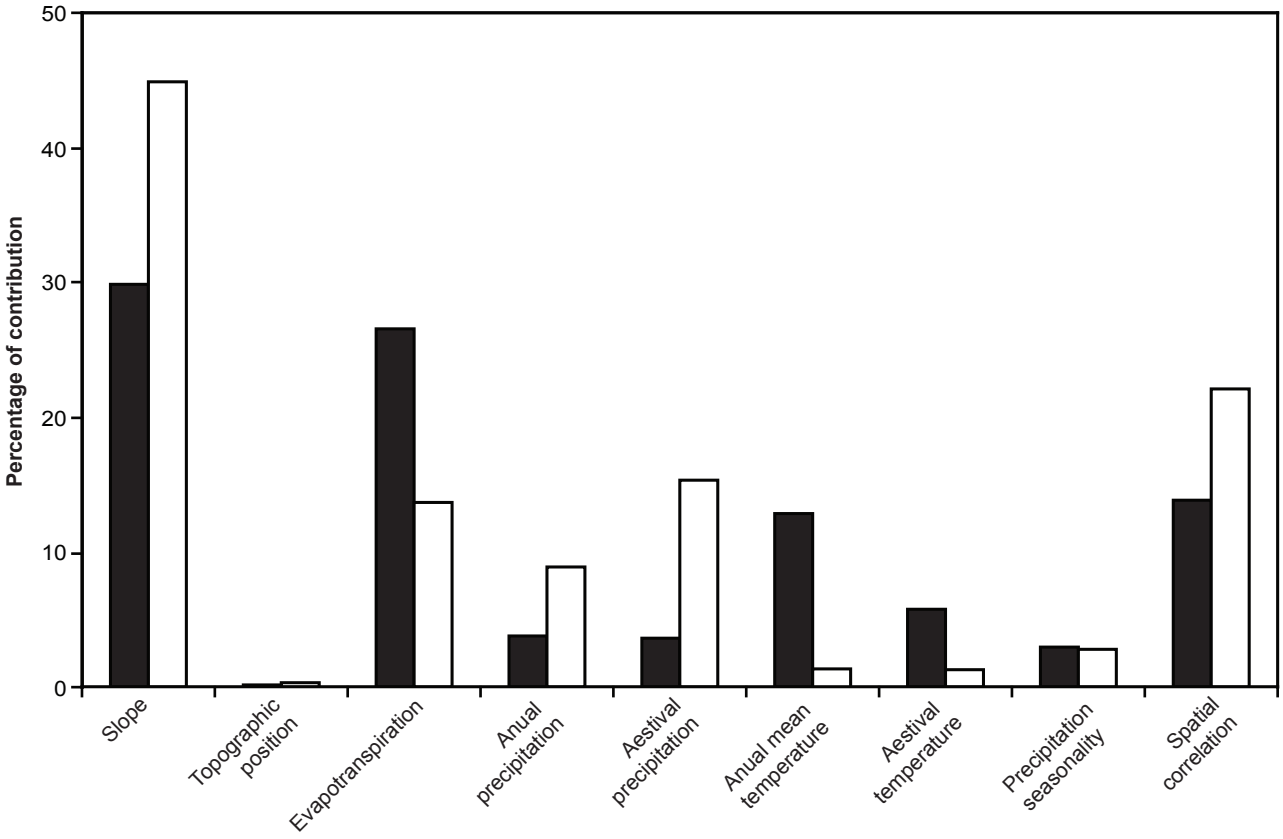


Figure 5. Contributions of environmental factors, given as the percentage of the total explained variance based on hierarchical partitioning analysis. Black bars represent independent contributions. White bars represent joint contributions.

with the exceptions of valleys formed by landslides. Therefore, it is unlikely that the species after landslides was present along the northern coast of Tenerife and then extinguished by human activity. A continuous distribution prior to landslides and recent eruptions would have required topographic conditions similar to those of the palaeoislands. These geomorphological conditions may have existed in geological times along the northern slope of the island before the landslides and eruptions events occurred. However, the potential distribution predicted by the models designates intermediate suitable areas where populations might have persisted until human colonisation, facilitating the connection between Teno and Anaga. The extirpation of these populations, together with a restricted seed dispersal mechanism, may have hindered genetic exchange between the disjunct populations in the last 100,000 years. An ongoing research with molecular markers, the study of bird pollination as a vector for gene flow and the effect of lizards on seed dispersal will contribute to clarify and validate the insights obtained in this work. On the other hand, further development of palaeoecological or climatic reconstructions (e.g. Nogué *et al.*, 2013) and climate change scenarios to project SMD will contribute to explain biogeographical patterns in this island.

REFERENCES

Ablay G.J. & Hürlimann M. (2000) Evolution of the north flank of Tenerife by recurrent giant landslides. *Journal of Volcanology and Geothermal Research*, **103**, 135–159.

Ancochea E. (2004) Canarias y el vulcanismo neógeno peninsular. *Geología de España* (ed. by J.A. Vera), pp. 635–680. SGE-IGME, Madrid.

Anderson M.J., Gribble N.A., & Cribble N. (1998) Partitioning the variation among spatial, temporal and environmental components in a multivariate data set. *Australian Journal of Ecology*, **23**, 158–167.

Aranda S.C. & Lobo J.M. (2011) How well does presence-only-based species distribution modelling predict assemblage diversity? A case study of the Tenerife flora. *Ecography*, **34**, 31–38.

Araùjo M.B. & Guisan A. (2006) Five (or so) challenges for species distribution modelling. *Journal of Biogeography*, **33**, 1677–1688.

del Arco Aguilar M.-J. (2006) *Mapa de vegetación de Canarias.* La Laguna, Tenerife.

del Arco Aguilar M.J., González-González R., Garzón-Machado V., & Pizarro-Hernández B. (2010) Actual and potential natural vegetation on the Canary Islands and its conservation status. *Biodiversity and Conservation*, **19**, 3089–3140.

Austin M.P. & Van Niel K.P. (2011) Improving species distribution models for climate change studies: variable selection and scale. *Journal of Biogeography*, **38**, 1–8.

Bañares-Baudet Á., Blanca G., Güemes J., Moreno-Saiz J.C., & Ortiz S. (2004) *Atlas y Libro Rojo de la Flora Vascular Amenazada de España.* Dirección General de Conservacón de la Naturaleza, Madrid.

Breiman L. (2001) Random forests. *Machine learning*, **45**, 5–32.

Brown R.P., Hoskisson P. a, Welton J.-H., & Báez M. (2006) Geological history and within-island diversity: A debris avalanche and the Tenerife lizard *Gallotia galloti*. *Molecular Ecology*, **15**, 3631–3640.

Carine M.A., Humphries C.J., Guma I.R., Reyes-Betancort J.A., & Santos Guerra A. (2009) Areas and algorithms: Evaluating numerical approaches for the delimitation of areas of endemism in the Canary Islands archipelago. *Journal of Biogeography*, **36**, 593–611.

Carine M.A., Russell S.J., Santos-Guerra A., & Francisco-Ortega J. (2004) Relationships of the Macaronesian and Mediterranean floras: Molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). *American Journal of Botany*, **91**, 1070–1085.

Carmen Machado Yanes M., Carmen del Arco Aguilar M., Del Arco Aguilar M.D.C., Vernet J.-L.L., & Ourcival J.-M.M. (1997) Man and vegetation in northern Tenerife (Canary Islands, Spain), during the prehispanic period based on charcoal analyses. *Vegetation history and archaeobotany*, **6**, 187–195.

Carrascal L.M., Seoane J., Palomino D., & Polo V. (2008) Explanations for bird species range size: Ecological correlates and phylogenetic effects in the Canary Islands. *Journal of Biogeography*, **35**, 2061–2073.

Carrete M., Grande J.M., Tella J.L., Sánchez-Zapata J.A., Donázar J. a., Díaz-Delgado R., & Romo A. (2007) Habitat, human pressure, and social behavior: Partialling out factors affecting large-scale territory extinction in an endangered vulture. *Biological Conservation*, **136**, 143–154.

Caujapé-Castells J. (2011) Jesters, red queens, boomerangs and surfers: a molecular outlook on the diversity of the Canarian endemic flora. *The biology of islands floras* pp. 284–

324. Cambridge University Press, Cambridge.

Caujapé-Castells J., Tye A., Crawford D.J., Santos-Guerra A., Sakai A., Beaver K., Lobin W., Vincent Florens F.B.B., Moura M., Jardim R., Gómes I., & Kueffer C. (2010) Conservation of oceanic island floras: Present and future global challenges. *Perspectives in Plant Ecology, Evolution and Systematics*, **12**, 107–129.

Chefaoui R.M. & Lobo J.M. (2008) Assessing the effects of pseudo-absences on predictive distribution model performance. *Ecological Modelling*, **210**, 478–486.

Chevan A. & Sutherland M. (1991) Hierarchical partitioning. *American Statistician*, **45**, 90–96.

Diniz-Filho J.A.F. & Bini L.M. (2008) Macroecology, global change and the shadow of forgotten ancestors. *Global Ecology and Biogeography*, **17**, 11–17.

Elith J., Graham C.H., & Graham J.C. (2009) Do they? How do they? WHY do they differ? On finding reasons for differing performances of species distribution models. *Ecography*, **32**, 66–77.

Elith J., Leathwick J.R., & Hastie T. (2008) A working guide to boosted regression trees. *Journal of Animal Ecology*, **77**, 802–813.

Emerson B.C. & Oromí P. (2005) Diversification of the forest beetle genus *Tarphius* on the Canary Islands, and the evolutionary origins of island endemics. *Evolution*, **59**, 586–598.

Escobar García P., Schönswetter P., Fuertes Aguilar J., Nieto Feliner G., & Schneeweiss G.M. (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). *Molecular Phylogenetics and Evolution*, **50**, 226–39.

ESRI (2004) ArcGIS 9.0.

Fernández-Palacios J.M., De Nascimento L., Otto R., Delgado J.D., García-del-Rey E., Arévalo J.R., & Whittaker R.J. (2011) A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography*, **38**, 226–246.

Franklin J. (2010) *Mapping species distributions: spatial inference and prediction.* Cambridge University Press,

Franklin J., Davis F.W., Ikegami M., Syphard A.D., Flint L.E., Flint A.L., & Hannah L. (2013) Modeling plant species distributions under future climates: how fine scale do climate projections need to be? *Global Change Biology*, **19**, 473–83.

Gómez-Campo C. (1996) *Libro rojo de especies vegetales amenazadas de las Islas Canarias.* Viceconsejería de Medio Ambiente, Gobierno de Canarias, Santa Cruz de Tenerife.

Guisan A., Jr T.E., Hastie T., & Edwards T.C. (2002) Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling*, **157**, 89–100.

Guisan A. & Thuiller W. (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**, 993–1009.

Guisan A., Tingley R., Baumgartner J.B., Naujokaitis-Lewis I., Sutcliffe P.R., Tulloch A.I.T., Regan T.J., Brotons L., Mcdonald-Madden E., Mantyka-Pringle C., Martin T.G., Rhodes J.R., Maggini R., Setterfield S.A., Elith J., Schwartz M.W., Wintle B.A., Broennimann O., Austin M.P., Ferrier S., Kearney M.R., Possingham H.P., & Buckley Y.M. (2013) Predicting species distributions for conservation decisions. *Ecology Letters*, **16**, 1424–1435.

Heads M. (2008) Biological disjunction along the West Caledonian fault, New Caledonia: A synthesis of molecular phylogenetics and panbiogeography. *Botanical Journal of the Linnean Society*, **158**, 470–488.

Heads M. & Craw R. (2004) The Alpine Fault biogeographic hypothesis revisited. *Cladistics*, **20**, 184–190.

Heikkinen R.K., Luoto M., Virkkala R., & Rainio K. (2004) Effects of habitat cover, landscape structure and spatial variables on the abundance of birds in an agricultural-forest mosaic. *Journal of Applied Ecology*, **41**, 824–835.

Hengl T., Sierdsema H., Radovika., & Dilo A. (2009) Spatial prediction of species' distributions from occurrence-only records: combining point pattern analysis, ENFA and regression-kriging. *Ecological Modelling*, **220**, 3499–3511.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hortal J., Lobo J.M., & Jiménez-Valverde A. (2007) Limitations of biodiversity databases: Case study on seed-plant diversity in Tenerife, Canary Islands. *Conservation Biology*, **21**, 853–863.

Hürlimann M., Marti J., & Ledesma A. (2004) Morphological and geological aspects related to large slope failures on oceanic islands: The huge La Orotava landslides on Tenerife, Canary Islands. *Geomorphology*, **62**, 143–158.

Irl S.D.H., Harter D.E.V., Steinbauer M.J., Gallego Puyol D., Fernández-Palacios J.M., Jentsch A., & Beierkuhnlein C. (2015) Climate vs. topography - spatial patterns of plant species diversity and endemism on a high-elevation island.

Journal of Ecology, **106**, 1621-1633.

Irl S.D.H., Steinbauer M.J., Messinger J., Blume-Werry G., Palomares-Martínez Á., Beierkuhnlein C., & Jentsch A. (2014) Burned and devoured-Introduced herbivores, fire, and the endemic flora of the high-elevation ecosystem on La Palma, Canary Islands. *Arctic, Antarctic, and Alpine Research*, **46**, 859–869.

Jestrow B., Gutiérrez Amaro J., & Francisco-Ortega J. (2012) Islands within islands: A molecular phylogenetic study of the *Leucocroton* alliance (Euphorbiaceae) across the Caribbean Islands and within the serpentinite archipelago of Cuba. *Journal of Biogeography*, **39**, 452–464.

Kier G., Kreft H., Lee T.M., Jetz W., Ibisch P.L., Nowicki C., Mutke J., Barthlott W., & Tien M.L. (2009) A global assessment of endemism and species richness across island and mainland regions. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 9322–9327.

Kumar L., Skidmore A.K., & Knowles E. (1997) Modelling topographic variation in solar radiation in a GIS environment. *International Journal of Geographical Information Science*, **11**, 475–497.

Lassueur T., Joost S., & Randin C.F. (2006) Very high resolution digital elevation models: Do they improve models of plant species distribution? *Ecological Modelling*, **198**, 139–153.

Li W., Guo Q., & Elkan C. (2011) Can we model the probability of presence of species without absence data? *Ecography*, **34**, 1096–1105.

Llanes P., Munoz A., Munoz-Martin A., Acosta J., Herranz P., Carbo A., Palomo C., ZEE W.G., Muñoz a., Muñoz-Martín a., & Carbó a. (2003) Morphological and structural analysis in the Anaga offshore massif, Canary Islands: fractures and debris avalanches relationships. *Marine Geophysical Researches*, **24**, 91–112.

Mairal M., Sanmartín I., Aldasoro J.J., Culshaw V., Manolopoulou I., & Alarcón M.L. (2015) Paleo-islands as refugia and sources of genetic diversity within volcanic archipelagos: The case of the widespread endemic *Canarina canariensis* (Campanulaceae). *Molecular Ecology*, **24**, 3944–3963.

McDowall R.M. (2008) Process and pattern in the biogeography of New Zealand - A global microcosm? *Journal of Biogeography*, **35**, 197–212.

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington DC.

Moreno-Saiz J.C. (2008) *Lista Roja de la flora vascular española*. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid.

Moya O., Contreras-Díaz H.G., Oromí P., & Juan C. (2004) Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). *Molecular Ecology*, **13**, 3153–3167.

Mac Nally R. & Walsh C.J. (2004) Hierarchical partitioning public-domain software. *Biodiversity and Conservation*, **13**, 659–660.

De Nascimento L., Willis K.J., Fernández-Palacios J.M., Criado C., & Whittaker R.J. (2009) The long-term ecology of the lost forests of la Laguna, Tenerife (Canary Islands). *Journal of Biogeography*, **36**, 499–514.

Nogué S., de Nascimento L., Fernández-Palacios J.M., Whittaker R.J., & Willis K.J. (2013) The ancient forests of La Gomera, Canary Islands, and their sensitivity to environmental change. *Journal of Ecology*, **101**, 368–377.

Oksanen J., Blanchet F.G., & Kindt R. (2010) vegan: community ecology package. .

Olesen J.M. & Valido A. (2003) Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution*, **18**, 177–181.

Perry G.H., Louis E.E., Ratan A., Bedoya-Reina O.C., Burhans R.C., Lei R., Johnson S.E., Schuster S.C., & Miller W. (2013) Aye-aye population genomic analyses highlight an important center of endemism in northern Madagascar. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 5823–8.

Peterson A.T. (2011) *Ecological niches and geographic distributions (MPB-49)*. Princeton University Press,

Pradervand J.-N., Dubuis A., Pellissier L., Guisan A., & Randin C.F. (2013) Very high resolution environmental predictors in species distribution models: Moving beyond topography? *Progress in Physical Geography*, **28**, 79–96.

Price J.P. (2004) Floristic biogeography of the Hawaiian Islands: influences of area, environment and paleogeography. *Journal of Biogeography*, **31**, 487–500.

Price J.P. & Clague D.A. (2002) How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings. Biological sciences / The Royal Society*, **269**, 2429–35.

Quinn G.P. & Keough M.J. (2002) *Experimental design and data analysis for biologists*. Cambridge.

R Core Team (2013) R: A Language and Environment for Statistical Computing. Version 3.1.2.

Rando J.C., Cabrera V.M., Larruga J.M., Hernández M., González A.M., Pinto F., Bandelt H.J., Hernández M., & González a M. (1999) Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. *Annals of Human Genetics*, **63**, 413–428.

Reyes-Betancort J.A., Santos Guerra A., Guma I.R., Humphries C.J., & Carine M. a. (2008) Diversity, rarity and the evolution and conservation of the Canary Islands endemic flora. *Anales del Jardín Botánico de Madrid*, **65**, 25–45.

Rodríguez Núñez S., Acevedo Rodríguez A., Oval de la Rosa J.P., Matos J., Rodríguez Martín B., & Rodríguez Martín A. (2004) Lavatera phoenicea Vent. *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (ed. by Á. Bañares-Baudet, G. Blanca, J. Güemes, J.C. Moreno, and S. Ortiz), pp. 895. Dirección General de Conservación de la Naturaleza, Madrid.

Seisdedos J., Ferrer M., & González de Vallejo L.I. (2012) Geological and geomechanical models of the pre-landslide volcanic edifice of Güímar and La Orotava mega-landslides (Tenerife). *Journal of Volcanology and Geothermal Research*, **239-240**, 92–110.

Solana M.C. & Aparicio A. (1999) Reconstruction of the 1706 Montaña Negra eruption. Emergency procedures for Garachico and El Tanque, Tenerife, Canary Islands. *Geological Society, London, Special Publications*, **161**, 209–216.

Swets J.A. (1988) Measuring the accuracy of diagnostic systems. *Science*, **240**, 1285–1293.

Tate J.A. & Fuertes Aguilar J. (2005) Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoideae) as inferred from ITS sequence data. *American Journal of Botany*, **92**, 584-602.

Thuiller W., Lafourcade B., Engler R., & Araùjo M.B. (2009) BIOMOD - a platform for ensemble forecasting of species distributions. *Ecography*, **32**, 369–373.

Trusty J.L., Olmstead R.G., Santos Guerra A., Sá-Fontinha S., Francisco-Ortega J., Santos-Guerra A., & Sá-Fontinha S. (2005) Molecular phylogenetics of the Macaronesian-endemic genus *Bystropogon* (Lamiaceae): palaeo-islands, ecological shifts and interisland colonizations. *Molecular Ecology*, **14**, 1177–1189.

Vanderpoorten A., Rumsey F.J., & Carine M.A. (2007) Does Macaronesia exist? Conflicting signal in the bryophyte and pteridophyte floras. *American Journal of Botany*, **94**, 625–639.

VanDerWal J., Shoo L.P., Graham C., & Williams S.E. (2009) Selecting pseudo-absence data for presence-only distribution modeling: How far should you stray from what you know? *Ecological Modelling*, **220**, 589–594.

Walter T.R., Troll V.R., Cailleau B., Belousov a., Schmincke H.-U.U., Amelung F., & Bogaard P. (2005) Rift zone reorganization through flank instability in ocean island volcanoes: An example from Tenerife, Canary Islands. *Bulletin of Volcanology*, **67**, 281–291.

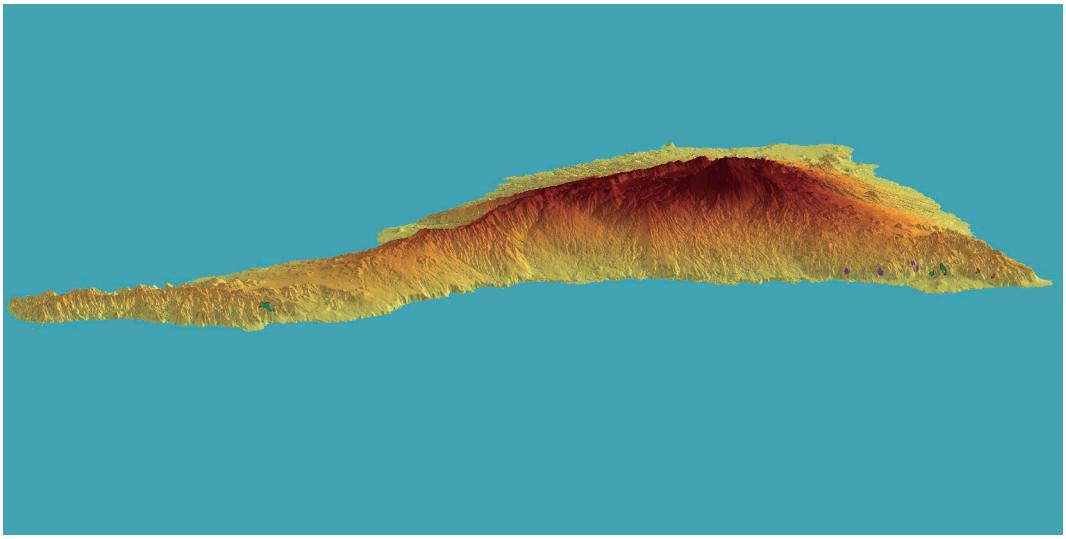
Whittaker R.J., Araùjo M.B., Paul J., Ladle R.J., Watson J.E.M., Willis K.J., & Jepson P. (2005) Conservation Biogeography: assessment and prospect. *Diversity and Distributions*, **11**, 3–23.

Whittaker R.J., Ladle R.J., Araùjo M.B., Fernández-Palacios J.M., Delgado J.D., & Arévalo J.R. (2007) The island immaturity - speciation pulse model of island evolution: an alternative to the "diversity begets diversity" model. *Ecography*, **30**, 321–327.

Zimmermann N.E., Edwards T.C., Graham C.H., Pearman P.B., & Svenning J.-C.C. (2010) New trends in species distribution modelling. *Ecography*, **33**, 985–989.

2 Congruence between spatial population genetics and bird pollination movements in gene flow estimation in populations of a Macaronesian endemic

Coherencia entre la estructura genética y los movimientos de las aves polinizadores en la estimación de flujo génico en las poblaciones de un endemismo Macaronésico



2. Congruence between spatial population genetics and bird pollination data in gene flow estimation in populations of a Macaronesian endemic

Congruence between spatial population genetics and bird pollination data in gene flow estimation in populations of a Macaronesian endemic

ABSTRACT

Spatial arrangements of genotypes and genetic diversity in insular taxa are determined by a number of biotic and biogeographic constraints but specific knowledge remains unclear. The surveyed flora of the Canary Islands shows high genetic diversity levels but the role of biotic constraints remains unexplored. In this paper we aimed to assess the contribution of biotic and abiotic factors to gene flow, particularly in relation to a threatened bird-pollinated plant species, the relict *Navaea phoenicea*, exclusive to Tenerife (Canary Islands) and restricted to the oldest volcanic massif cores, Anaga (East) and Teno (West). We specifically focused on the effect of geographical barriers in promoting population differentiation, and the genetic homogenization effect of the bird pollination system. For that purpose we combined an AFLP marker analysis with observation of flower visitors and tracking of pollen analogues. We sampled 304 individuals in 8 subpopulations in Anaga and 7 in Teno. N. phoenicea showed a high genetic diversity within all subpopulations, as well as high levels of genetic introgression in 7 out of 8 detected genetic clusters. Spatial Genetic Structure was detected at different scales in Teno and Anaga, and was higher in the first population. Only one out of 2 bird species frequently visiting N. phoenicea revealed itself as an effective pollinator (Canarian chiffchaff, Phylloscopus canariensis). Its spatial visitation patterns were consistent with genetic differences among subpopulations and gene flow, and contribute to explaining present genetic structure. This study also shows that further molecular ecology research in oceanic insular systems should incorporate spatial arrangement of populations and animal plant interactions beyond the classical historical biogeographical approach.

2. Congruence between spatial population genetics and bird pollination data in gene flow estimation in populations of a Macaronesian endemic

INTRODUCTION

Genetic diversity and population structure in plants are the result of past and present environmental processes, including human influence and evolutionary drivers. Oceanic islands have been classical models for biogeographical studies and offer particular environments for research on the interplay of major determinants of spatial genetic structure such as dispersal, gene flow and spatial distribution of individuals (Whittaker & Fernández-Palacios, 2007). Even if oceanic islands are young, they harbour a diverse mosaic of areas of different age. This is as consequence of their geological dynamism and the continuous process of eruptions, emergences, landslides and subsidences (Stuessy *et al.*, 2012), that determine the spatial arrangement of genetic diversity. At the same time, the distribution of populations is strongly conditioned by microclimatic factors, determined by the local topography, and to a great extent, by the anthropization of ecosystems. Also, although oceanic barriers often hinder gene flow between islands and continental sources (Emerson, 2002), and it is often considered that insular biota may face the founder effects, many other factors drive very different levels of genetic variation (Stuessy *et al.*, 2013), not necessarily lower (e.g. García-Verdugo *et al.*, 2015)

The Canary Islands are situated 100 km away from the north-western Sahara coast (28ºN) and are a well-known example of this complexity of factors. The archipelago emerged from the East to the West across a linear axis with a large diversity in island ages (Anguita & Hernán, 2000). To date, molecular studies on Canary Islands taxa have primarily focused on conservation and phylogeography (Francisco-Ortega *et al.*, 2000; Caujapé-Castells *et al.*, 2010; Caujapé-Castells, 2011). Nevertheless, there is some contradictory evidence on the average levels of genetic diversity and differentiation of the Canarian flora (Francisco-Ortega *et al.*, 2000; Caujapé-Castells, 2009, 2011). De Paz and Caujapé (2013) provided evidence of the effect of several biogeographical and life history traits on genetic variation such as ploidy level (see Besnard *et al.*, 2008; García-Verdugo *et al.*, 2009), reproductive system, population size and dispersal. However ecological processes shaping genetic structure or fine-scale spatial genetic structure (SGS), i.e, the non-random distribution of genotypes across short space scales (Vekemans & Hardy, 2004), have been explored only for few species.

Navaea phoenicea (Vent.) Webb & Berthel. ("Malva de risco encarnada", Malvaceae) is a large-sized, hexaploid shrub endemic to Tenerife Island. The singular life history of this species together with environmental determinants of geographic distribution and ecological interactions, make this species a good model to test the impact of several insular traits on genetic diversity and population genetic structure.

Firstly, geological history is likely to play a role in the population structure of *Navaea*. Within the Canarian archipelago, Tenerife is geologically the most complex island, comprising the three ancient volcanic shields of Anaga (East), Teno (East) and Roque del Conde (South), connected by eruptions during the last 3 Ma (Ancochea, 2004). Subsequent structural instabilities generated episodes of massive landslides during the last 800,000 years (Hürlimann *et al.*, 2004; Boulesteix *et al.*, 2013) conforming the current geomorphology. There is already evidence that such geological complexity has impacted on the population genetics of the insular biota since landslides are known to alter the genetic structure and demographic dynamics of insects, lizards or plants, and constitute a significant barrier between populations (Moya *et al.*, 2004; Brown *et al.*, 2006; Bloor *et al.*, 2008; Macías-Hernández *et al.*, 2013; Mairal *et al.*, 2015). On the other hand, islands have been subject of a deep human perturbation , but leaving areas in the old massifs better conserved (del Arco Aguilar *et al.*, 2010). *Navaea phoenicea* presents this disjunct distribution pattern in Tenerife, with one population in Anaga and one in Teno, composed both by several subpopulations. The combination of niche restriction, geological processes and anthropic fragmentation in the Teno-Anaga disjunction of this species (chapter 1) may preclude gene flow and enhance SGS.

Secondly, *Navaea phoenicea* represents a Tertiary relict maintained in the Canarian archipelago (Tate *et al.*, 2005; Escobar García *et al.*, 2009). The Macaronesian region is considered to some extent a refuge for genetic diversity (Vargas, 2007; García-Verdugo *et al.*, 2009; Fernández-Mazuecos & Vargas, 2011), while at the same time, narrow distributions may be associated with limited genetic diversity (García-Verdugo *et al.*, 2013).

Thirdly, *Navaea phoenicea* is a threatened species considered as endangered (EN) according to the IUCN categories and subject to progressive habitat loss and population decline (Rodríguez Núñez *et al.*, 2004; Moreno-Saiz, 2008) which can lead to a low genetic diversity.

Fourthly, *Navaea phoenicea* is a hexaploid species and it is predominantly outcrossing, since delayed selfing mechanisms present in other mallows are not efficient (chapter 4). Polyploidy has been assumed to provide a higher genetic diversity (Soltis & Soltis, 2000) as a result of an increase in the number of genes, although it is often associated to asexual reproduction (Brochmann *et al.*, 2004; Guldahl *et al.*, 2005). The influence of polyploidy on genetic diversity has received recent attention only for a scarce number of taxa in the Canary Islands (Besnard *et al.*, 2008; García-Verdugo *et al.*, 2009, 2013). Within the Malvaceae family this question remains fairly unexplored as well (Small & Wendelt, 2002; Wallace *et al.*, 2005).

Finally, gene flow mediated by pollen and seed dispersal depends on mating system and animal interactions, determining the levels of genetic variation, population structure and the occurrence of SGS. In this context, *N. phoenicea* belongs to the so-called "Macaronesian Bird-flower Element", an ensemble of Macaronesian endemics with common floral traits adapted to bird pollination (Olesen, 1985; Olesen & Valido, 2004). Four generalist passerines have been identified as potential pollinators: Canarian chiffchaff (*Phylloscopus canariensis*), Canarian blue tit (*Cyanistes teneriffae*), Blackcap warbler (*Sylvia atricapilla*) and Sardinian warbler (*Sylvia melanocephala*). Field assessments of pollination efficiency determined that *C. teneriffae* is an illegitimate disruptor nectar robber whilst *Sylvia spp.* are infrequent pollinators, thus leaving *P. canariensis* as the most efficient pollinators (chapter 4). It is frequently assumed that gene flow is higher in bird pollinated species than in insect pollinated species, but consequences on genetic structure of pollination by vertebrates have received little attention (Krauss *et al.*, 2009). On the other hand, the common lizard *Gallotia gallotii* (Lacertidae) is an infrequent seed disperser and may contribute to extend gene flow as observed for other Canarian taxa (Rumeu *et al.*, 2011). However since seed dispersal occurs mostly by barochory, narrow gene flow can be predicted from this trait (Vekemans & Hardy, 2004; Dick *et al.*, 2008).

Rare or endemic species are more likely to be self-compatible, have lower reproductive investment, and lower genetic diversity than closely related common species (Lavergne *et al.*, 2004). However, other endemic species exhibit outcrossing and have relatively high levels of genetic diversity (Jabis *et al.*, 2011). Conversely, the

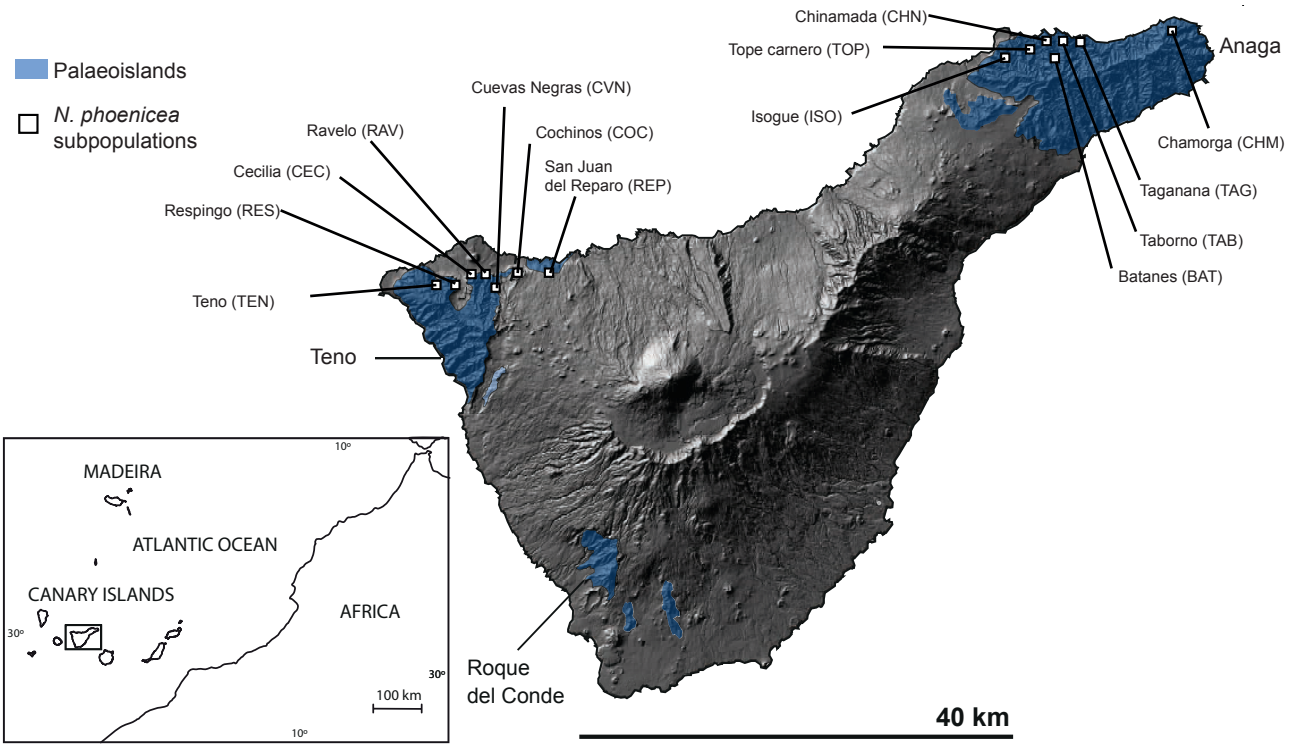


Figure 1. Location of the study area within the Canarian Archipelago and distribution of *Navaea phoenicea* populations. Blue areas denote the present palaeoislands in Tenerife.

Table 1. Sampled subpopulations, UTM coordinates and genetic diversity indices Ho (Observed heterozygosity), H_{SH} (Nei's index), P (polymorphic loci), Hs=Shannon index.

Population	Subpopulation	UTM Coordinates	Nº of samples	Ho	H_{SH}	P	H_S
Anaga	Batanes	373391 3159234	12	0.262	0.247	0.616	0.360
	Carboneras	374938 3160920	16	0.156	0.1587	0.498	0.2414
	Chamorga	386661 3161628	27	0.303	0.2599	0.638	0.3779
	Chinamada	373241 3160579	7	0.304	0.3053	0.782	0.4475
	Isogue	369836 3158486	36	0.309	0.3187	0.793	0.4636
	Taganana	379279 3159709	8	0.269	0.2588	0.715	0.3821
	Taborno	375853 3160477	10	0.272	0.2827	0.727	0.4138
	Tope carnero	372112 3159782	15	0.278	0.2699	0.671	0.3926
Mean Anaga				**0.269**	**0.262**	**0.680**	**0.384**
Teno	Cecilia	321141 3137625	8	0.264	0.2807	0.704	0.4090
	Cochinos	322398 3137456	15	0.248	0.2380	0.575	0.3444
	Cuevas Negras	320789 3138494	42	0.261	0.2754	0.668	0.3985
	Ravelo	327561 3139003	11	0.269	0.2811	0.712	0.4102
	Reparo	316524 3137706	67	0.246	0.2298	0.572	0.3339
	Respingo	316935 3137459	14	0.216	0.2187	0.560	0.3212
	Teno	316453 3137410	48	0.231	0.2538	0.664	0.3725
Mean Teno				**0.247**	**0.254**	**0.636**	**0.370**
Total mean				**0.259**	**0.259**	**0.660**	**0378**

species do not show the predicted autogamy resulting from natural selection from pollinator limitation in small isolated or low-density populations.

This assemble of factors affecting population genetic constitution in *N. phoenicea* raises the overarching question of whether bird pollination and polyploidy can overcome relictual character, low number of individuals and geographical barriers to ensure species viability. By taking *N. phoenicea* as the case study we assessed spatial genetic structure, gene flow and its relationship with bird pollination, and investigated genetic signals of potential past/successive events of population reduction and bottlenecks. Specifically, we aimed to test the following hypotheses: 1) geographical barriers interrupt gene flow, therefore gene flow between populations should be limited; 2) genetic variation is lower than the average for the Canarian endemic flora 3) SGS is detectable at the population level, but possibly also within subpopulations due to a high degree of differentiation among populations, and 4) generalist passerines visiting *N. phoenicea* flowers can act as gene flow vectors beyond population boundaries. AFLPs markers and quantitative analysis of bird movements were used to test these hypotheses.

MATERIALS AND METHODS

Sampling and DNA extraction

Sampling was constrained by the difficult access to populations. Fresh young leaves were collected from 304 accessible georeferenced individuals of *N. phoenicea* subpopulations (7 in Teno and 8 in Anaga), spanning the whole species distribution (table 1, figure 1), and dried and stored with silica gel. A small number of seedlings, constrained by official permissions, was also taken for subsequent paternity analyses. Genomic DNA was extracted from dried leaves using DNeasy Plant Minikit (QIAGEN) modifying manufacturer's instructions to improve precipitation of secondary metabolites. Concentration of the isolated DNA was determined by spectrophotometry. Quality was determined by 1.5% Tris-Acetate-EDTA (TAE)-agarose electrophoresis. Sybr-safe staining with negative controls was performed to monitor contamination.

AFLP protocols and fragment analysis

We followed the procedure described by Vos *et al.* (1995), with an initial screening of 32 combinations of selective primers on eight individuals, 4 from Teno and 4 from Anaga. Four combinations producing clear peaks with selective primers labelled with FAM fluorochromes were selected. Since conventional protocols based on 3 selective

2. Congruence between spatial population genetics and bird pollination data in gene flow estimation in populations of a Macaronesian endemic

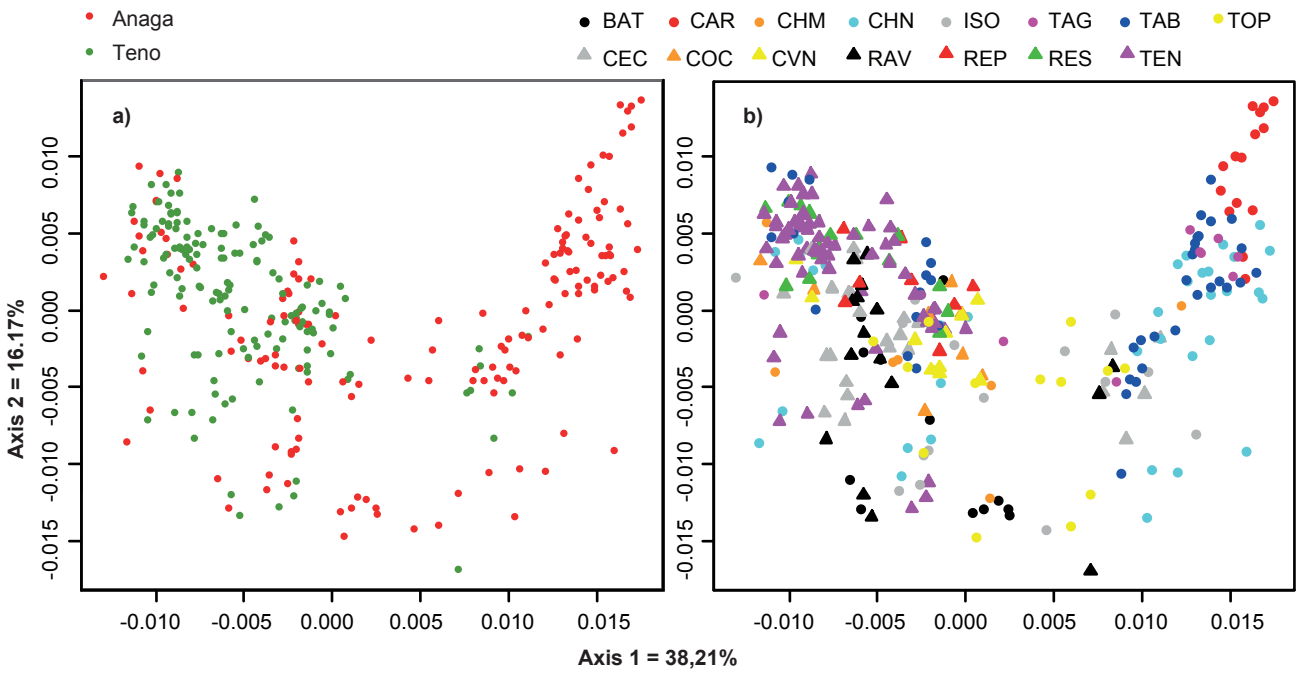


Figure 2. Principal coordinates analysis results for axis 1 and 2. a) individuals classified by populations, in b) by subpopulations.

bases in selective amplification yielded an excessive number of peaks, we used 4 selective bases (CATG, CAGC, CTCC, CTAG). To avoid mismatching, preselective amplifications were done with 2 selective bases. The fragments were separated using an ABI PRISM 3700 sequencer (Applied Biosystems) using 1 µL of polymerase chain reaction (PCR) product and GeneScan-500 LIZ size standard.

Amplified bands were automatically sized with a LIZ internal size standard using Genemapper 3.0 (Applied Biosystems, 2004), and then manually corrected. Peaks were recorded in a range from 150 to 600 bp. Scoring was made through Bayesian analysis with the AFLPscore package (Whitlock *et al.*, 2008) implemented in the R software. A reproducibility test was performed by re-extracting DNA from 24 individuals in the 15 populations, and repeating the complete procedure. The threshold for peak validation was 150 Relative Fluorescence Units which minimized both mismatching and type II errors. Fragments within the 0-150 bp range were dismissed, in order to minimize overlapping of non-homologous peaks and those that failed to be reproduced in the test.

Table 2. Pairwise FST distances computed for subpopulations. All results in statistically significant different after 10000 permutations test except those values highlighted in bold.

	BAT	CAR	CEC	CHM	CHN	COC	CVN	ISO	RAV	REP	RES	TAB	TAG	TEN
CAR	0.2391													
CEC	0.0864	0.2224												
CHM	0.0646	0.2026	0.0866											
CHN	0.1096	0.1395	0.1015	0.0842										
COC	0.0844	0.2229	0.0846	0.0682	0.1045									
CVN	0.0828	0.2143	0.0824	0.0697	0.0992	**0**								
ISO	0.058	0.1758	0.0731	0.0552	0.0417	0.0603	0.0576							
RAV	0.0649	0.221	**0**	0.0731	0.0976	0.0759	0.0761	0.0526						
REP	0.1165	0.2245	0.106	0.0717	0.1153	0.0417	0.0273	0.0723	0.0966					
RES	0.1444	0.2542	0.0946	0.0933	0.152	0.0931	0.0939	0.1061	0.0865	0.069				
TAB	0.1404	0.1555	0.1086	0.0879	0.0845	0.1133	0.1065	0.075	0.1141	0.1143	0.1347			
TAG	0.1047	0.1189	0.0913	0.0606	0.0585	0.0794	0.0666	0.0506	0.0857	0.0839	0.1197	**0**		
TEN	0.1475	0.2884	0.1044	0.1131	0.1726	0.1207	0.1191	0.1246	0.0979	0.1063	0.0023	0.1578	0.15	
TOP	0.0707	0.1897	0.0896	0.0765	0.0807	0.0862	0.0802	**0**	0.0697	0.1048	0.1343	0.0914	0.0639	0.1508

Genetic diversity and differentiation

The following genetic diversity indexes were calculated for each subpopulation assuming HW equilibrium: 1) proportion of polymorphic loci (P); 2) observed heterozygosity (H_o); 3) Shannon diversity index (H_S, Shannon, 1948) using POPGENE 1.32 (Yeh *et al.*, 1997) 4) Nei's unbiased gene diversity index (HSH, Nei, 1978) assuming Hardy-Weinberg equilibrium using TFPGA 1.3 (Miller, 1997). A descriptive comparison of AFLP phenotypes was made through the calculation of pairwise Nei's similarity matrix between all individuals using the Dice coefficient and the construction of a Neighbour-Joining tree in R software. A Principal Coordinates Analysis (PCoA) was performed with the Ade4 package in R software (Jombart, 2008) based on Nei's genetic distance D (Nei, 1972) between individuals.

Population genetic differentiation and structure

To explore genetic clustering of individuals we employed two techniques based on Markov Chain Monte Carlo Bayesian methods implemented in STRUCTURE 2.3 (Hubisz *et al.*, 2009) and BAPS 6.0 (Corander *et al.*, 2004). In STRUCTURE, we used the admixture model with correlated allele frequencies. We ran 10 simulations of 10^7 iterations with a burning period of 10^6 and visually confirmed the convergence of chains. Simulations were run for a number of clusters K=1-15. We applied the ΔK statistic described by Evanno *et al.* (2005) with Structure Harvester program (Earl & von Holdt, 2012). The program CLUMPP 1.1. (Jakobsson & Rosenberg, 2007) was used to combine the independent runs to produce a single probability of membership for each individual for the optimal value of K. The program *DISTRUCT* 1.1 (Rosenberg, 2004) was used to plot individual membership probabilities. For BAPS, we also chose an admixture model with sampling localities information and K values also ranged from 1-15 with 10 runs for each K.

To estimate the degree of genetic differentiation among subpopulations, we estimated pairwise F_{ST} (Wright, 1951) with AFLPsurv software (Vekemans, 2002). To explore the partitioning of genetic variance between populations and between Teno and Anaga nuclei, we used Arlequin 3.3 (Excoffier *et al.*, 2005) to perform an AMOVA test considering three levels: within subpopulations, between subpopulations in each population and between populations. We performed two runs considering two different groupings, Teno-Anaga, and the 8 genetic clusters determined by BAPS and STRUCTURE (see results).

Spatial Genetic Structure was evaluated using the software SPAGeDi (Hardy & Vekemans, 2002). We selected the F_{ij} coefficient (Hardy, 2003) for dominant markers. Correlograms of F_{ij} against distance classes and *Sp* statistic where built following Vekemans and Hardy (2004) for Teno and Anaga populations. The significance of the observed regression slope log-bf was tested using 10,000 permutations. Wright's neighbourhood size N_b and historical gene dispersal (sigma-g) were also computed as effective population density had been estimated previously as 0.30.

In addition, to test the correlation between geographic and distance matrices, we analysed the pairwise genetic and geographic distances by means of a Mantel test at the interpopulation level. The analysis was performed with the Adegenet package (Jombart, 2008) using 100,000 permutation tests for the level of significance.

For a small number of seedlings we conducted paternity analyses using FAMOZ (Gerber *et al.*, 2003). Among the collected seedlings we selected those that met the following requirements: 1) to be located within 1.5 meters from a reproductive individual, which we considered as the mother plant; and 2) not having any other reproductive individual within less than 6 meters, assigning the mother plant to each seedling in the least ambiguous manner.

Table 3. Hierarchical analysis of molecular variance (AMOVA) based on allelic variation at two geographical levels: (a) between Teno and Anaga **populations and (b) between genetic clusters determined by Bayesian methods**

	d.f	Sum of squares	Variance components	% Var	P	F
a) Teno vs. Anaga						
Among populations	1	699.38	3.13	7.16	<0.001	Fct=0.07157
Among subpopulations (pops)	13	1815.436	5.71	13.04	<0.001	Fsc=0.14048
Within subpopulations	289	10100.4	34.94	79.8	<0.001	
b) 8 genetic clusters						
Among clusters	7	2207.15	7.28	17	<0.001	Fct=0.170
Among subpopulations (clusters)	7	397.664	0.61	1.43	<0.001	Fsc=0.0.017
Within subpopulations	289	10100.4	34.94	81.57	<0.001	

Logarithm of the odds (LOD scores) were calculated following Krauss *et al.* (2009). We considered as the most likely father the individual with best LOD and Delta scores, allowing a 5% error threshold.

Pollen dispersal and pollinators movements

We carried out pollinator censuses over 4 flowering seasons (2005-2009), encompassing around 4 months each (October-January). In total, flower visitations by insects and birds were monitored for 8 subpopulations each season. Pollinator movements were observed by means of: 1) direct daily observation of every single visitor movement between flowers of individuals within a population one at a time. From a discrete position where all the population could be observed with binoculars, we accounted throughout daily sessions all the visits performed by each individual. Spatial position of flowers had been previously recorded, and this allowed to record the distance travelled

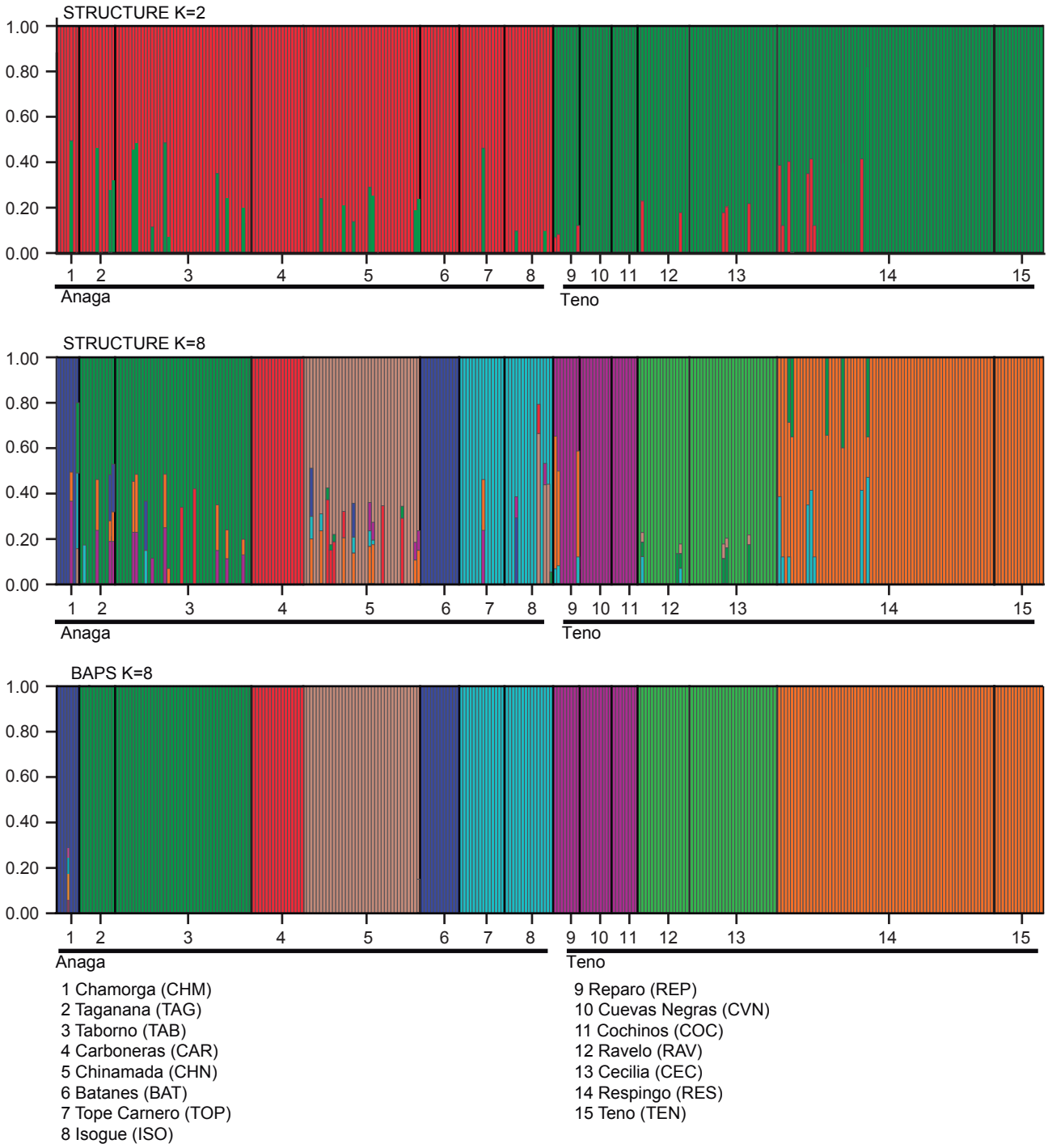


Figure 3. Assignment of the sampled individuals of *Navaea phoenicea* to the K groups inferred by STRUCTURE and BAPS Bayesian algorithms. Every bar correspond to one individual, numbers correspond to the populations ordered as in Table 1.

Table 4. Results for SGS analyses performed with Spagedi: bF-log: logarithm of regression slope, Sp statistic, **Nb, Wright's neighbourghood size, and σ, gene dispersal coefficient.**

Population	bF-log	Sp	Nb	σ
Teno	-0.022	0.021	28.37	2.14
Anaga	-0.023	0.022	39.57	2.53

between visited flowers; 2) Over three flowering seasons, we applied fluorescent powder to 4 different flowers at the anthesis stage with 4 different colours before dawn every day, and after dusk we examined the flowers as well as the neighbour subpopulations with black light in search of fluorescent traces. We allowed a three day time lag between successive powder applications at each nuclei to allow the fluorescent powder to disappear.

RESULTS

AFLP profiles

Highly reproducible AFLP patterns were obtained for all 24 replicates subjected in the reproducibility test. 150 RFU as threshold minimized mismatching error below 3.7% and type II error below 1.4%. 15 fragments were not replicated and were discarded. The reproducibility error at the AFLP phenotype level was 0.5%. In total, we obtained a data set of 271 loci, from which 20 were monomorphic bands. Neither identical phenotypes nor privative alleles were found.

Genetic diversity and differentiation

Genetic diversity indices for geographical populations are shown in table 1. Diversity scores remained high across all populations. The lowest indices corresponded to the Carboneras population (P=0.498, H_{SH}=0.1587, H_o=0.156, and H_s=0.2414), while the Isogue population showed the highest (P=0.793, H_{SH}=0.3187, H_o=0.309, and H_s=0.4636). Overall, the Anaga massif showed higher genetic diversity than Teno.

In the PCoA analysis the first three axes accounted for 68.51% of the variance (A1=38.21%, A2=16.17%, and A3=14.13%, figure 2). These results show a visible separation between Teno and Anaga groups (figure 2a), but differentiation was much less evident regarding geographical populations (figure 2b), suggesting admixture between populations. Similarly, STRUCTURE output provided evidence for gene flow between subpopulations. ΔK provided support for K=2 and K=8 (figure 3a and b). BAPS was coincident in K=8 (figure 3c). Although genetic clusters are coherent with geographical distribution of populations, STRUCTURE showed two significant results: on the one hand, strong cluster admixture was observed not only among clusters but interestingly, also between Teno and Anaga regions. On the other hand, three populations in Anaga showed no admixture, despite the absence of geographic isolation: Carboneras remained fully isolated in one cluster and Batanes and Chamorga, which is the most geographically isolated population, conformed another. Only Chamorga had a slight admixture with Teno clusters. In the same manner, the NJ tree based on pairwise F_{ST} distances (figure 4, table 2) showed a split between Teno and Anaga with the exception of Chamorga-Batanes cluster. CAR population appeared to be joined to CHN, but showed in turn the highest genetic distance. AMOVA analysis (table 3) ascribed the largest share of variance to the within-population component. AMOVA showed more variance assigned between populations than between subpopulations (7.09% versus 3.18% for Teno-Anaga

Table 5. Paternity assignment of seedlings and corresponding mother and best father selected according to LOD score. Dist= distance between **best father and mother; LOD= Logarithm of the Odd score value; Loci=number of loci that with a contribution >0 to LOD score; Mismatch=number of mismatch loci between father and seedling; Nº of fathers= number of putative fathers identified by FaMoz. Asterisks show fathers from a different subpopulation.**

Seedling	Mother plant	Best father	Dist	LOD	Loci	Mismatch	Nº of fathers
CHN113	CHN111	CHN904	440	31.73	265	5	4
CEC303	CEC203	CEC107	9.5	47.42	271	4	2
TEN311	TEN310	TEN311	0	31.16	271	3	4
TEN427	TEN417	RES110*	0	25.79	271	8	4
TOP04	TOP01	ISO121*	2200	42.49	269	9	5
TAB14	TAB54	TAB02	70	44.8	271	1	1
Mean distance	**450**	**Ssd**	**870**				

structuring and 18.63% versus 1.67% for the 8 clusters detected by Bayesian clustering) indicating the existence of gene flow between Teno and Anaga. Allocated variance between groups was higher when considering detected genetic clusters than Teno-Anaga nuclei (18.63% versus 7.09%).

Fine-scale Spatial Genetic Structure

Individual Mantel tests performed for the ensemble of Teno and Anaga populations supported a significant relationship between geographic distance and genetic differentiation ($p<0.0001$), but with low correlation indices ($R^2=0.63$). Kinship analyses detected a significant negative regression slope (figure 5, table 5) up to the 11th distance class. *Sp* showed very similar values in Teno and Anaga (*Sp*=0.022), but with different patterns of SGS. Anaga population showed a non-significant value for F_{ij} for the first class and a subsequent decrease until the 4th class (20 meters). On the contrary, F_{ij} values decreased constantly with distance in Teno population. Wright's neighborhood sizes inferred from SGS were 38 for Anaga and 51 for Teno.

Paternity analysis

A total of six seedlings in compliance with the exclusion criteria were found (table 5). The paternity exclusion probability was high (EP=0.998). Simulation tests using FaMoz gave a threshold likelihood ratio value of 2.50. Using this threshold, maximum likelihood assignment matched the six offsprings with a most-likely sire within the ensemble of sampled individuals in both populations. Two out of six assigned fathers came from the closest subpopulation. Mean distance between best father and mother was 450 m, but distances varied greatly from two (nearest neighbor) to 2200 meters.

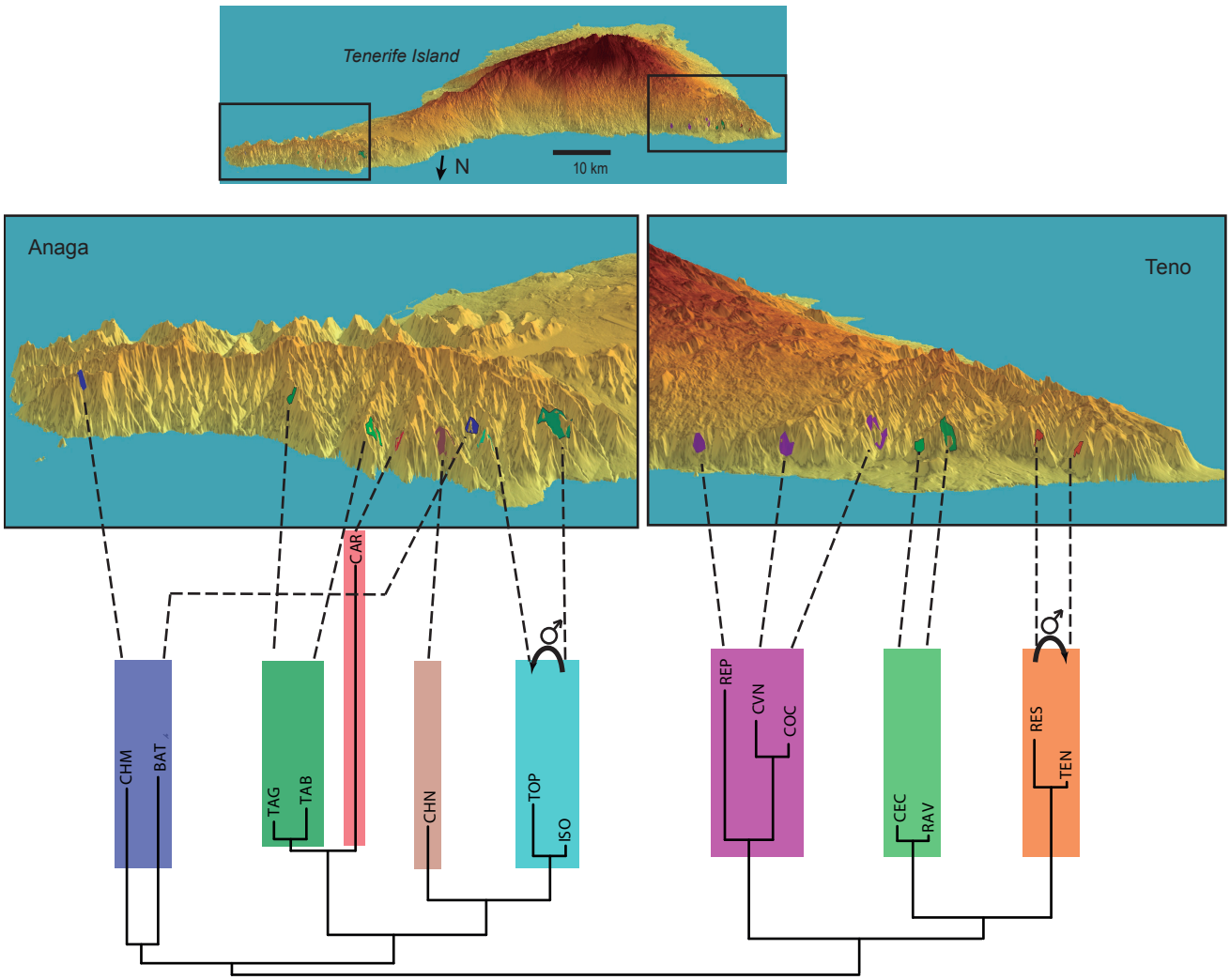


Figure 4. Unrooted neighbor-joining tree for the 15 subpopulations and the 271 AFLP loci. This tree is based on pairwise differences among the AFLP patterns obtained using Nei and Li distance. Subpopulations appear in the topographic map in the same order as in the tree except for Batanes (BAT). Arrows indicate subpopulations where seedlings paternity test indicate gene flow via pollen between subpopulation. The direction of the arrow differentiates source of pollen and subpopulation.

Pollinator movements

Bird visitations to flowers were dominated by *P. canariensis* (42%) and *C. teneriffae* (39%). The two warbler species accounted only for 9% of the bird visits. *P. canariensis* were the visitors travelling the longest distances between flowers (table 6a, ANOVA, P=0.001). The amount of consecutive visits to flowers of the same individual plant (i.e., geitonogamy) was significantly lower in chiffchaffs as they traveled greater distances. Conversely, blue tits accounted for a larger number of geitonogamic visits which led to lower distances travelled between flowers.

The use of fluorescent powder as pollen analogue revealed the extension of the movements of birds, beyond the mere observation in a single spot. In total 192 flowers with fluorescent powder were detected. On average, particles travelled 64.50 meters with a high variance in the values, ranging from the closest flower within an individual (i.e., geitonogamy), to movements to the neighbor subpopulation 2022 meters away. Pollen movements beyond the subpopulation boundaries were found to happen in eight out of 15 populations (table 6b).

DISCUSSION

This is the first study conducted in the Canary Islands directly relating pollinator movements to gene flow and genetic structure. These results from population structure and AMOVA provide evidence of contemporaneous gene flow between both populations despite the magnitude of the geographical gap existing between them. Results derived from polymorphic markers and from the spatial pattern of movements found for bird species were highly consistent, which appear to allow the extension of gene flow beyond subpopulations limits. Pollinator mobility thus contributes significantly to overcome isolation by distance.

Genetic diversity and population structure of *Navaea phoenicea*

N. phoenicea exhibited a high level of genetic diversity despite its restricted range. Levels of genetic variation in AFLPs were within the range of other geographically restricted Canarian species (Kim *et al.*, 2005; Prohens *et al.*, 2007), but they were higher than other widely distributed tree species (García-Verdugo *et al.*, 2009, 2013). *Canarina canariensis*, which shows similar patterns of distribution within Tenerife and bird pollination syndrome, but greater population numbers and bird dispersal syndrome (González-Castro *et al.*, 2015), exhibited similar levels of genetic variation (Mairal *et al.*, 2015). While the review of the allozyme diversity for the Canarian flora (Pérez de Paz & Caujapé-Castells, 2013) failed to predict a significant effect of pollination ecology, here pollinators may contribute to increase genetic diversity through pollen exchange between subpopulations. The AFLP analysis provided two kinds of evidence for this and are discussed in detail below. The first one is that results show that gene flow through pollination is frequently extended beyond one subpopulation, and may contribute to overcome such geographic disjunction The second evidence refers specifically to the values of lower genetic diversity detected for the subpopulation of Carboneras which are discussed below on the basis of genetic differentiation and can be related directly to the role of pollinator vectors.

Table 6. a) Results of visitor censuses to the flowers of *Navaea phoenicea* averaged for the whole period of the study;
b) Records of pollen analogues brushed in a source subpopulation and found in another subpopulation (destination).

a)

Species	% of visits	Distance traveled between visits	% of geitonogamic visits
P. canariensis	42	27.4 ± 36.87	16%
C. teneriffae	39	11.29 ± 14.15	87%
S. melanocephala	4	11.86 ± 31.86	17%
S. conspicilliata	5	11.25 ± 22.02	19%

b)

Subpopulation source	Destination	Number of ocurrences	Distance (meters)
ISO	TOP	3	1878
CHN	ISO	1	2022
COC	CVN	1	1051
CVN	COC	3	1051
RAV	CEC	3	695
TEN	RES	1	916

Both PCoA analyses and the NJ tree showed a clear but incomplete split between Teno and Anaga and an unclear differentiation between subpopulations. Genetic clustering determined by STRUCTURE and BAPS showed eight genetic groups consistent with its spatial distribution across the northern coast, but with remarkable considerations. The first one is the high level of genetic introgression among clusters, which is especially important between Teno and Anaga, and indicates the persistence of a certain amount of gene flow in spite of the Teno-Anaga disjunction. AMOVA results consistently allocated a small share of genetic variance between populations, that is, between Teno and Anaga. In chapter 1, ecological suitable areas for *Navaea phoenicea* were detected across the center of the island. This possible presence of intermediate populations in ravines between Teno and Anaga could have facilitated potential gene flow until their destruction in recent times by human land occupation. The second consideration is the notable absence of genetic introgression in Carboneras and Batanes subpopulations despite the absence of geographic isolation. Carboneras provides interesting evidence for gene flow processes, since its flowering period was significantly advanced and showed little overlap with the other subpopulations (chapter 3) which may be due to be located at the warmest spot. Therefore, local climatic constraints may determine local reproductive isolation through a slight phenological differentiation which precludes pollen flow between neighbor subpopulations. Batanes is related to Chamorga subpopulation, but they are not geographically near and only the latter is geographically isolated. The genetic relationship may be interpreted as a recent colonization from a long distance dispersal event, as individuals are located in a former cultivated terrace currently under secondary succession. Alternatively, as a result of the volcanic activity affecting palaeoislands, both subpopulations are the only ones located in the same dyke extrusion (Marinoni & Gudmundsson, 2000).

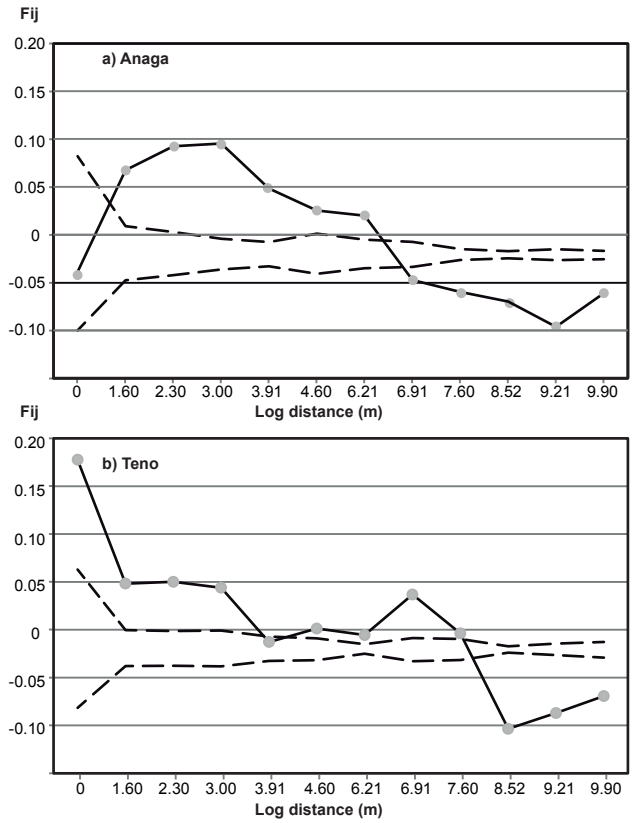


Figure 5. Correlograms for Spatial Genetic Structure (SGS) of the populations of Teno and Anaga versus logarithm of the distance. Continuous line represents Fij values calculated for each distance class, dashed lines represent the 95% CI limits for the permutations.

There is empirical support for the fact that geological processes constitute frequently a more effective barrier than oceanic ones, at least in the Canary Islands (García-Verdugo *et al.*, 2014; Mairal *et al.*, 2015). Here, although this hypothesis in not tested, both genetic structure but also niche modeling provide evidence supporting a disjunct distribution from geological origin as well, followed by a plausible scenario of isolated intermediate populations which could serve as bridge, facilitating gene flow through the geographic gap.

Spatial genetic structure of populations

SpaGeDi results provided evidence for SGS at both populations in spite of the extent of pollen flow. In particular analyses showed relatively high *Sp* statistic (0.021 – 0.022). These values are partly in line with those reviewed by Vekemans & Hardy (2004) for plant traits; in particular small tree life form (0.0259) and reproductive systems, *Sp* fell in between mixed mating (0.0372) and outcrossing (0.0126). What could drive SGS to such extent? Low density and strong aggregation of individuals at short distances are likely causes for SGS pattern (Vekemans & Hardy, 2004), but gene flow may balance a localized barochory proccess. There were two remarkable differences between Teno and Anaga. Spatial correlation was not significant in Anaga at the shortest distant, but so was in Teno. Anaga in turn showed a higher value for Wright's neighbourhood size. This difference points to a more localized seed dispersal at short distances in Teno but not in Anaga. The secondary, infrequent seed dispersal event by the common lizard *Gallotia gallotia* may be higher in Anaga. The slightly higher σ values obtained for Anaga

also support gene flow differences between populations.

Despite that the Canary Islands provide an excellent opportunity to test the effect of particular mutualistic interactions, studies assessing fine-scale spatial genetic structure in this archipelago are scarce (López de Heredia *et al.*, 2010; Saro *et al.*, 2014) and comparisons are difficult to make. Given the amount of plant traits related to insularity which may be relevant for the levels of SGS, it is necessary to incorporate better spatial information to genetic studies to account for the drivers of genetic structure in islands. Other studies for instance have been able to account for the effect of bird movements and the extent of seed dispersal in insular plants depending on the fruit traits (Theim *et al.*, 2014).

Evidences for gene flow mediated by floral visitors

Direct and indirect assessment of pollen flow provided strong evidence for consistency between gene flow among detected clusters, genetic diversity and spatial structure. First, our results showed different distances traveled by each flower visitor. *P. canariensis* and *C. teneriffae* showed the same visitation rates and they were far more frequent than warblers, but only the first of the two species acted as a legitimate pollinators. Distances travelled by *P. canariensis* between flowers were abnormally high and showed a low frequency of geitonogamic events. In turn, the spatial visitation patterns of the species when visiting *Isoplexis canariensis* consisted in shorter visits to neighbor plants, optimizing the energy balance between foraging behavior and nectar intake (Rodríguez-Rodríguez *et al.*, 2015). It is therefore intriguing what factors drive such differences in spatial movements. One explanation may be that the energy balance as a result of feeding from *N. phoenicea* nectar can compensate the energetic expenses of longer displacements. Second, consistency between the pattern of visits by *P. canariensis* and gene flow can also be demonstrated by FaMoz paternity results despite the constrained sample. All the scanned surveyed seedlings were assigned to outcrossing, while for two of them the assigned parents came from the nearest subpopulations. Third, pollen analogues provided evidence for pollen flow among subpopulations, as distances travelled by pollen analogues are coincident with the spatial pattern observed by chiffchaffs within subpopulations. These findings suggest that pollinators can visit two different subpopulations within three days, while the long lasting fertility of pollen grains (up to eight days, chapter 3) allows cross pollination to occur. Despite a deep analysis of the pollination ecology of *N. phoenicea*, we did not find any direct evidence of ringed chiffchaffs in other subpopulations.

Conclusions

Despite an evident geographic barrier for gene flow and its narrow distribution, *N. phoenicea* showed a high level of genetic variation and certain gene flow mediated by pollinators. This is the first molecular approach to disentangle the effect of pollinators on gene flow within the ensemble of life history traits that determine genetic structure and diversity in a Macaronesian palaeoendemic, and to assess SGS and paternity analyses. The combination with SDMs (chapter 1) provided insights of past events and spatial distributions explaining ancestral patterns of gene flow. The increasing body of research on islands regarding ecological processes such as pollination and seed dispersal mechanisms should open a whole new research field where biogeographical and ecological differences between continents and islands can contribute to understand spatial population genetics.

REFERENCES

Ancochea E. (2004) Canarias y el vulcanismo neógeno peninsular. *Geología de España* (ed. by J.A. Vera), pp. 635–680. SGE-IGME, Madrid.

Anguita F. & Hernán F. (2000) The Canary Islands origin: a unifying model. *Journal of Volcanology and Geothermal Research*, **103**, 1–26.

Applied Biosystems (2004) Genemapper. 1.0.

del Arco Aguilar M.J., González-González R., Garzón-Machado V., & Pizarro-Hernández B. (2010) Actual and potential natural vegetation on the Canary Islands and its conservation status. *Biodiversity and Conservation*, **19**, 3089–3140.

Besnard G., García-Verdugo C., De Casas R.R., Treier U.A., Galland N., & Vargas P. (2008) Polyploidy in the olive complex (*Olea europaea*): evidence from flow cytometry and nuclear microsatellite analyses. *Annals of Botany*, **101**, 25–30.

Bloor P., Kemp S.J., & Brown R.P. (2008) Recent volcanism and mitochondrial DNA structuring in the lizard *Gallotia atlantica* from the island of Lanzarote. *Molecular Ecology*, **17**, 854–866.

Boulesteix T., Hildenbrand A., Soler V., Quidelleur X., & Gillot P.-Y. (2013) Coeval giant landslides in the Canary Islands: Implications for global, regional and local triggers of giant flank collapses on oceanic volcanoes. *Journal of*

Volcanology and Geothermal Research, **257**, 90–98.

Brochmann C., Brysting A.K., Alsos I.G., Borgen L., Grundt H.H., Scheen A.C., & Elven R. (2004) Polyploidy in arctic plants. *Biological Journal of the Linnean Society*, **82**, 521–536.

Brown R.P., Hoskisson P. a, Welton J.-H., & Báez M. (2006) Geological history and within-island diversity: A debris avalanche and the Tenerife lizard Gallotia galloti. *Molecular Ecology*, **15**, 3631–3640.

Caujapé-Castells J. (2009) General GST and h inflation due to biased intra-population sampling, and its consequences for the conservation of the Canarian Flora. *Conservation Genetics*, **11**, 709–720.

Caujapé-Castells J. (2011) Jesters, red queens, boomerangs and surfers: a molecular outlook on the diversity of the Canarian endemic flora. *The biology of islands floras* pp. 284–324. Cambridge University Press, Cambridge.

Caujapé-Castells J., Tye A., Crawford D.J., Santos-Guerra A., Sakai A., Beaver K., Lobin W., Vincent Florens F.B.B., Moura M., Jardim R., Gómes I., & Kueffer C. (2010) Conservation of oceanic island floras: Present and future global challenges. *Perspectives in Plant Ecology, Evolution and Systematics*, **12**, 107–129.

Corander J., Waldmann P., Marttinen P., & Sillanpää M.J. (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, **20**, 2363–2369.

Dick C.W., Hardy O.J., Jones F.A., & Petit R.J. (2008) Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology*, **1**, 20–33.

Earl D.A. & von Holdt B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Emerson B.C. (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology*, **11**, 951–966.

Escobar García P., Schönswetter P., Fuertes Aguilar J., Nieto Feliner G., & Schneeweiss G.M. (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). *Molecular Phylogenetics and Evolution*, **50**, 226–39.

Evanno G., Regnaut S., & Goudet J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L., Laval G., & Schneider S. (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Informatics Online*, **1**, 47–50.

Fernández-Mazuecos M. & Vargas P. (2011) Genetically depauperate in the continent but rich in oceanic islands: *Cistus monspeliensis* (Cistaceae) in the Canary Islands. *PLoS ONE*, **6**, e17172.

Francisco-Ortega J., Santos-Guerra A., Kim S.C., & Crawford D.J. (2000) Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany*, **87**, 909–919.

García-Verdugo C., Baldwin B.G., FAY M.F., & Caujapé-Castells J. (2014) Life history traits and patterns of diversification in oceanic archipelagos: A meta-analysis. *Botanical Journal of the Linnean Society*, **174**, 334–348.

García-Verdugo C., Calleja J.A., Vargas P., Silva L., Moreira O., & Pulido F. (2013) Polyploidy and microsatellite variation in the relict tree *Prunus lusitanica* L.: how effective are refugia in preserving genotypic diversity of clonal taxa? *Molecular Ecology*, **22**, 1546–1557.

García-Verdugo C., Fay M.F., Granado-Yela C., De Casas R.R., Balaguer L., Besnard G., & Vargas P. (2009) Genetic diversity and differentiation processes in the ploidy series of Olea europaea L.: a multiscale approach from subspecies to insular populations. *Molecular Ecology*, **18**, 454–67.

García-Verdugo C., Sajeva M., La Mantia T., Harrouni C., Msanda F., & Caujapé-Castells J. (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. *Molecular Ecology*, **24**, 726–741.

Gerber S., Chabrier P., & Kremer A. (2003) FAMOZ: a software for parentage analysis using dominant, codominant and uniparentally inherited markers. *Molecular Ecology Notes*, **3**, 479–481.

González-Castro A., Calviño-Cancela M., & Nogales M. (2015) Comparing seed dispersal effectiveness by frugivores at the community level. *Ecology*, **96**, 808–818.

Guldahl A.S., Gabrielsen T.M., Scheen A.C., Borgen L., Steen S.W., Spjelkavik S., & Brochmann C. (2005) The *Saxifraga rivularis* complex in Svalbard: Molecules, ploidy and morphology. *Flora*, **200**, 207–221.

Hardy O.J. (2003) Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. *Molecular Ecology*, **12**, 1577–1588.

Hardy O.J. & Vekemans X. (2002) SPAGeDI: A versatile computer program to analyse spatial genetic structure at

the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hubisz M.J., Falush D., Stephens M., & Pritchard J.K. (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**, 1322–1332.

Hürlimann M., Marti J., & Ledesma A. (2004) Morphological and geological aspects related to large slope failures on oceanic islands - The huge La Orotava landslides on Tenerife, Canary Islands. *Geomorphology*, **62**, 143–158.

Jabis M.D., Ayers T.J., & Allan G.J. (2011) Pollinator-mediated gene flow fosters genetic variability in a narrow alpine endemic, *Abronia alpina* (Nyctaginaceae). *American Journal of Botany*, **98**, 1583–94.

Jakobsson M. & Rosenberg N.A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Jombart T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.

Kim S.C., Lee C., & Santos-Guerra A. (2005) Genetic analysis and conservation of the endangered Canary Island woody sow-thistle, *Sonchus gandogeri* (Asteraceae). *Journal of Plant Research*, **118**, 147–153.

Krauss S.L., He T., Barrett L.G., Lamont B.B., Enright N.J., Miller B.P., & Hanley M.E. (2009) Contrasting impacts of pollen and seed dispersal on spatial genetic structure in the bird-pollinated *Banksia hookeriana*. *Heredity*, **102**, 274–285.

Lavergne S., Thompson J.D., Garnier E., & Debussche M. (2004) The biology and ecology of narrow endemic and widespread plants: a comparative study of trait variation in 20 congeneric pairs. *Oikos*, **107**, 505–518.

López de Heredia U., Venturas M., López R.A., & Gil L. (2010) High biogeographical and evolutionary value of Canary Island pine populations out of the elevational pine belt: The case of a relict coastal population. *Journal of Biogeography*, **37**, 2371–2383.

Macías-Hernández N., Bidegaray-Batista L., Emerson B.C., Oromí P., & Arnedo M. (2013) The imprint of geologic history on within-island diversification of woodlouse-hunter spiders (Araneae, Dysderidae) in the canary islands. *Journal of Heredity*, **104**, 341–356.

Mairal M., Sanmartín I., Aldasoro J.J., Culshaw V., Manolopoulou I., & Alarcón M.L. (2015) Paleo-islands as refugia and sources of genetic diversity within volcanic archipelagos: The case of the widespread endemic *Canarina canariensis* (Campanulaceae). *Molecular Ecology*, **24**, 3944–3963.

Marinoni L.B. & Gudmundsson A. (2000) Dykes, faults and palaeostresses in the Teno and Anaga massifs of Tenerife (Canary Islands). *Journal of Volcanology and Geothermal Research*, **103**, 83–103.

Miller M.P. (1997) Tools for Population Genetic Analyses (TFPGA) 1.3: A Windows Program for the Analysis of the Allozymes and Molecular Genetic Data. .

Moreno-Saiz J.C. (2008) *Lista Roja de la flora vascular española.* Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid.

Moya O., Contreras-Díaz H.G., Oromí P., & Juan C. (2004) Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). *Molecular Ecology*, **13**, 3153–3167.

Nei M. (1972) Genetic Distance Between Populations. *The American Naturalist*, **106**, 283–&.

Nei M. (1978) Estimation of Average Heterozygosity and Genetic Distance from A Small Number of Individuals. *Genetics*, **89**, 583–590.

Olesen J.M. (1985) The Macaronesian bird-flower element and its relation to bird and bee oppurtunists. *Journal of the Linnean Society*, **91**, 395–414.

Olesen J.M. & Valido A. (2004) Lizards and birds as generalized pollinators and seed dispersers of island plants. *Insular Ecology* (ed. by J.M. Fernández Palacios and C. Morici), pp. 229–250. Asociación Española de Ecología Terrestre y Excmo.Cabildo Insular de La Palma, La Palma.

Pérez de Paz J. & Caujapé-Castells J. (2013) A review of the allozyme data set for the Canarian endemic flora: causes of the high genetic diversity levels and implications for conservation. *Annals of Botany*, **111**, 1059–73.

Prohens J., Anderson G.J., Herraiz F.J., Bernardello G., Santos-Guerra A., Crawford D.J., & Nuez F. (2007) Genetic diversity and conservation of two endangered eggplant relatives (*Solanum vespertilio* Aiton and *Solanum lidii* Sunding) endemic to the Canary Islands. *Genetic Resources and Crop Evolution*, **54**, 451–464.

Rodríguez Núñez S., Acevedo Rodríguez A., Oval de la Rosa J.P., Matos J., Rodríguez Martín B., & Rodríguez Martín A. (2004) *Lavatera phoenicea* Vent. *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (ed. by Á. Bañares-Baudet, G. Blanca, J. Güemes, J.C. Moreno, and S. Ortiz), pp. 895. Dirección General de Conservación de la

Naturaleza, Madrid.

Rodríguez-Rodríguez M.C., Jordano P., & Valido A. (2015) Hotspots of damage by antagonists shape the spatial structure of plant-pollinator interactions. *Ecology*, **96**, 2181–2191.

Rosenberg N.A. (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Rumeu B., Caujapé-Castells J., Blanco-Pastor J.L., Jaén-Molina R., Nogales M., Elias R.B., Vargas P., & Jaen-Molina R. (2011) The colonization history of *Juniperus brevifolia* (Cupressaceae) in the Azores islands. *PLoS ONE*, **6**, e27697.

Saro I., Robledo-Arnuncio J.J., González-Pérez M.Á., & Sosa P.A. (2014) Patterns of pollen dispersal in a small population of the Carianan endemic palm (*Phoenix canariensis*). *Heredity*, 1–9.

Shannon C.E. (1948) A Mathematical Theory of Communication. *Bell Systems Technical Journal*, **27**, 379–423.

Small R.L. & Wendelt J.F. (2002) Differential evolutionary dynamics of duplicated paralogous Adh loci in allotetraploid cotton (*Gossypium*). *Molecular Biology and Evolution*, **19**, 597–607.

Soltis P.S. & Soltis D.E. (2000) The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7051–7057.

Stuessy T.F., Takayama K., López-Sepúlveda P., & Crawford D.J. (2013) Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society*, **174**, 276–288.

Stuessy T.F., Takayama K., & Sepúlveda P.L. (2012) Founder effects are invisible in endemic species of oceanic islands. *Journal of Biogeography*, **39**, 1565–1566.

Tate J.A., Fuertes Aguilar J., Wagstaff S.J., La Duke J.C., & Bodo Slotta T.A. (2005) Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoidae) as inferred from ITS sequence data. *American Journal of Botany*, **92**, 584–602.

Theim T.J., Shirk R.Y., & Givnish T.J. (2014) Spatial genetic structure in four understory *Psychotria* species (Rubiaceae) and implications for tropical forest diversity. *American Journal of Botany*, **101**, 1189–1199.

Vargas P. (2007) Are Macaronesian islands refugia of relict plant lineages ? A molecular survey. *Phylogeography of Southern European refugia* pp. 297–314. Springer, The Netherlands.

Vekemans X. (2002) AFLPsurv. 1.0.

Vekemans X. & Hardy O.J. (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, **13**, 921–935.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters a, Pot J., Peleman J., & Kuiper M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic acids research*, **23**, 4407–14.

Wallace L.E., Helenrum K., & Helenurm K. (2005) Characterization of microsatellite loci in polyploid *Lavatera assurgentiflora* ssp. *assurgentiflora* and ssp. *glabra* (Malvaceae). *Molecular Ecology Notes*, **6**, 331–333.

Whitlock R., Hipperson H., Mannarelli M., Butlin R.K., & Burke T. (2008) An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Molecular Ecology Resources*, **8**, 725–735.

Whittaker R.J. & Fernández-Palacios J.M. (2007) *Island biogeography: ecology, evolution, and conservation.* Oxford University Press, Chippenham.

Wright S. (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

Yeh F.C., Yang R.-C., Boyle T.B.J., Ye Z.-H., & Mao J.X. (1997) POPGENE, the user-friendly shareware for population genetic analysis. 1.0.

3 Pollen limitation prevents self-pollination in a self-compatible palaeoendemic species

La limitación polínica impide la autofecundación en un paleoendemismo autocompatible

Pollen limitation prevents self-pollination in a self-compatible palaeoendemic species

ABSTRACT

Mating systems in plants are crucial traits for reproductive success and genetic diversity, shaped by evolutionary strategies. In volcanic archipelagos, this trait may be even more important as colonization success is highly challenged by the absence of other mates. In the Malvaceae family, the delayed-salting strategy is considered to be advantageous, but frequent exceptions have been found. We focused in studying the mating system of *Navaea phoenicea*, an ornithophilous mallow endemic to Tenerife (Canary Islands). We monitored flower phenology and assessed the basic floral traits: pollen fertility, P/O ratio, flowering stages, stigma receptivity and the effect of pollination on delayed selfing. We found a subpopulation with the flowering period slightly out of step of the general flowering synchrony. This may be due to a differentiated climate conditions that promote a flowering in advance. Pollen was fertile all along the life of the flower and stigmas were fertile. However, we observed a generalized failure of the delayed selfing mechanism, as pollen was shed when styles where exerted. Rather than a mechanism to ensure fertilization, herkogamy here may be a strategy to promote outcrossing. This has important consequences on both genetic diversity and reproductive success, especially considering that reproductive assurance rely then on opportunistic passerines rather than in specialist nectarivores.

INTRODUCTION

Plant mating systems are central for determining both the spatial and temporal patterns of genetic diversity within and between populations (Barrett *et al.*, 2003; Wright *et al.*, 2008; Karron *et al.*, 2012) and population demography and evolutionary processes (Lloyd, 1992; Wright *et al.*, 2008; Pannell, 2015). For hermaphroditic angiosperms, mating systems across families have shifted recurrently from complete outbreeding to complete self-fertilization, attracting a large body of theoretical research aiming to understand why selfing evolves from outcrossing (Barrett, 2004). Outbreeding is considered beneficial because it promotes genetic diversity and reduces inbreeding depression and the effects of deleterious genes. Self-incompatibility has been likewise shown as a driver for family diversification (Ferrer & Good, 2012), that has appeared recurrently across evolution of angiosperms but has beem frequently lost, often due to the loss of self-incompatible S locus (Igic *et al.*, 2008), although late self-incompatibility mechanisms have received minor attention (Gibbs, 2014). Selfing, including induced autogamy by pollinators and geitonogamy, is considered adaptive as it would ensure reproductive success (see Busch & Delph, 2012 for review), especially under animal visitors shortage (Eckert, 2000) or human disturbances (Eckert *et al.*, 2010). Therefore, reproductive assurance is the primary explanation for the evolution of selfing (Herlihy & Eckert, 2002). Intermediate (mixed) systems have been widely developed in plant families as a result of different ecological or genetic mechanisms (Goodwillie *et al.*, 2005). Mixed mating systems encompass the floral mechanisms to avoid either selfing or male-female interferences through temporal (dichogamy) or spatial (herkogamy) isolation of anthers and pistils. Therefore they can confer adaptive advantages by promoting outcrossing when pollinators are present, but allowing selfing when they are scarce or absent. For instance, dichogamy has been described as one mechanism that helps reducing both self-fertilization and interference between male and female sexual functions (Bertin & Newman, 1993). Within the dichogamuos systems, delayed selfing induced by floral movements allows facultative reproductive success when mates are scarce (Tsitrone *et al.*, 2003) without incurring in pollination discount (e.g. Kalisz, Vogler & Hanley, 2004).

The evolution and prevalence of different mating systems is an essential issue considered in insular ecology and biogeography (Anderson *et al.*, 2001; Pannell, 2015; Pannell *et al.*, 2015), because isolation affects both reproductive assurance and availability of pollinators. Mating systems that might be considered as adaptive in continental environments may not be favored by species dispersed to islands (Pannell, 2006). A plant establishing in a new territory by long distance dispersal such as invasive species or island colonization is challenged by the absence of mates, pollinator scarcity and inbreeding depression (Barrett, 2010). Therefore, island colonization success enforces a tradeoff between assuring reproductive success and promoting genetic exchange. The evolution of mating systems in insular flora has generated a large of research background (e.g. Carlquist, 1966), and it has been referred to as a model for testing the so-called "Baker's rule" (Baker, 1955) which postulates that self-compatible taxa are favoured in localities best colonized by long distance dispersal, where mates are scarce. to some extent support for Baker's rule has been found (Cheptou, 2012; Auld & Rubio de Casas, 2013), there is also a number of reported exceptions across oceanic archipelagos, where nevertheless studies on oceanic flora remain scarce (Crawford *et al.*, 2008). While the higher prevalence of self-compatible species in oceanic islands is a sustain for Baker's rule, the also higher occurrence of dioecy contradicts it (Pannell, 2006). Novel outcrosser taxa in oceanic islands also confront unpredictable or poor pollinator environment (Trøjelsgaard *et al.*, 2013; Castro-Urgal & Traveset, 2014), and their effectiveness may depend on the specialist or generalisation degree, which in turn depends on the age of the island (Trøjelsgaard *et al.*, 2013). For example, in the well-studied Canary Islands, allozyme diversity is higher in total or partial self-incompatible species (Pérez de Paz & Caujapé-Castells, 2013). Finally, ploidy level

Table 1. Subpopulations of *Navaea phoenicea* where experiments were carried out

Subpopulation	UTM Coordinates	Population	Subpopulation	UTM Coordinates	Population
Carboneras (CAR)	374938 3160920	Anaga	Cecilia (CEC)	321141 3137625	Teno
Chinamada (CHN)	373241 3160579	Anaga	Cuevas Negras (CVN)	320789 3138494	Teno
Taborno (TAB)	375853 3160477	Anaga	San Juan del Reparo (REP)	316524 3137706	Teno
Tope Carnero (TOP)	372112 3159782	Anaga	Teno (TEN)	316453 3137410	Teno

plays a significant role on the rate of self-fertilization, with polyploids showing greater rates than their diploid relatives (Barringer, 2007). Although polyploid taxa are not as frequent as in mainland flora, this condition may contribute to counter genetic erosion. Oceanic islands host a significant number of polyploidy taxa, often linked to insular arborescence. As newly formed polyploid faces reproductive isolation (Fowler & Levin, 1984), selection favours those traits that contribute to the reproductive success of the neopolyploid. Consequently, most polyploid plants possess means for asexual reproduction, which may confer the ability to delay reproductive efforts in the absence of a suitable mate (Stebbins, 1940; Gustafsson, 1948). Likewise a breakdown of self-incompatibility mechanisms frequently accompanies polyploidization, which prevents the need for a mate (Stebbins, 1957; Ramsey & Schemske, 1998). Stebbins (1950) noted that polyploidy in annual plants, which is uncommon, would invariably be restricted to those taxa that were self-compatible.

Navaea phoenicea (Vent.) Webb & Berthel. (Malvaceae) is an hexaploid, small tree or big-sized shrub exclusive from Tenerife (Canary Islands). As a relict species, it is basal to the Malveae tribe (Escobar García *et al.*, 2009) and classified as Endangered under IUCN categories (Moreno-Saiz, 2008). The Malvaceae family is a self-compatible family for most known species. In general terms, in the family the flowers' stamens are arranged in a single column encircling styles, which are exerted further above the staminal column towards the anthers (figure 1). Therefore, the mating strategy can be considered as either approach herkogamy from a spatial point of view, or, as protandry, from a temporal point of view. Ruan *et al.* (2010) differentiated that two types of curvature towards anthers which recurrently appear across Malvaceae tribes, likely related to different selection pressures. The main interpretation for stylar movements has been delayed selfing, but promotion of outcrossing and reduction of male-female interference are also considered hypotheses (Ruan *et al.*, 2010). Likewise, in several well studied species, pollination precludes style curvature (Ramsey *et al.*, 2003; Seed *et al.*, 2006; Ruan *et al.*, 2008, 2011), thus it may be considered an adaptive strategy associated to variable pollination environments. *Navaea phoenicea* belongs to the so-called "Macaronesian bird-flower element" (MBE), an ensemble of Macaronesian endemics presenting a bird-pollination syndrome (Olesen & Valido, 2004; Valido *et al.*, 2004) and visited by generalist passerines. Because this syndrome appears to be a relict condition in such endangered species, the pollination effectiveness of current flower visitors and the contribution of selfing to reproductive success is a fundamental element to acknowledge for conservation measures.

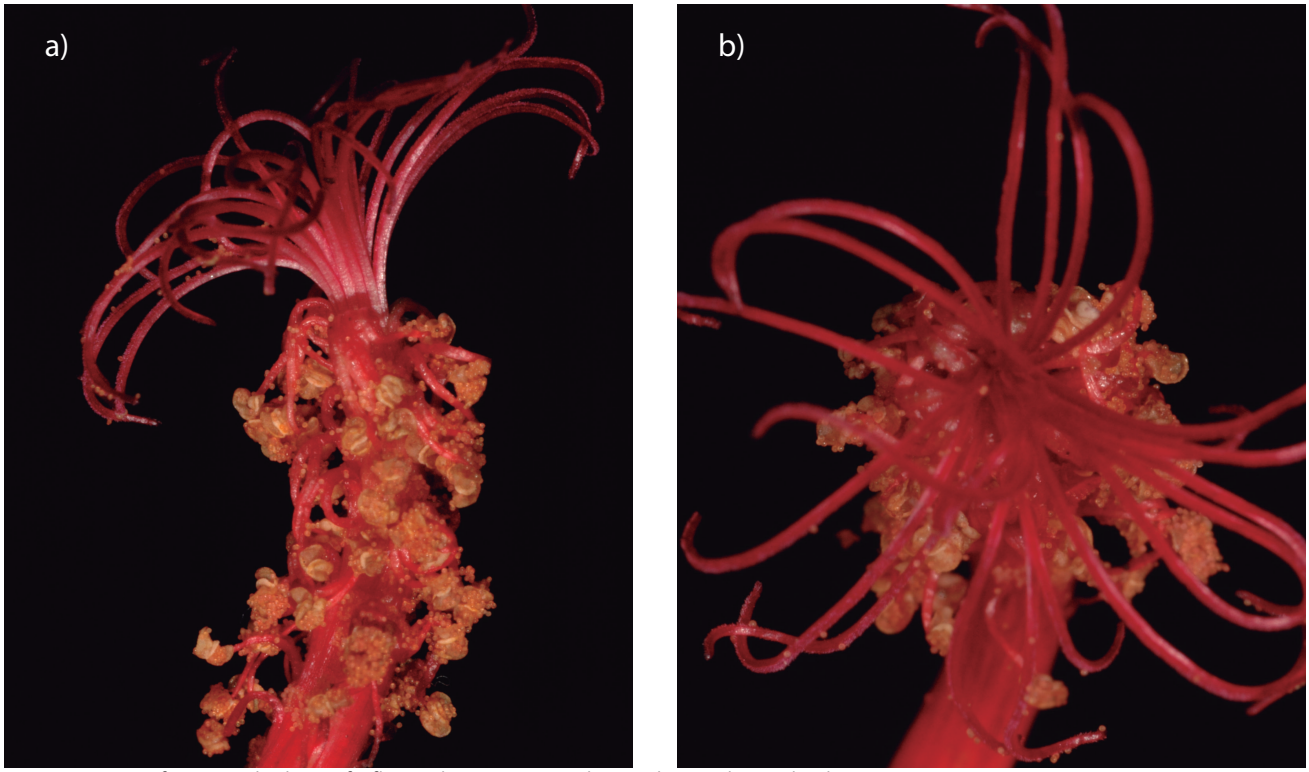


Figure 1. Aspect of a staminal column of a flower showing open anthers and exerted curved styles.

This insular endemic taxon represents therefore an extremely interesting model to study the evolution of mating system from the combined perspective of a) the evolution of protandry and self-fertilization within the Malvaceae family, and b) the evolution of insular biota. This combined approach allows us to discuss the role of selfing within the Macaronesian Bird-Flower element as well.

The specific aims of the present work were the following: 1) to ascertain several basic reproductive aspects from *Navaea phoenicea* reproductive biology (phenology, flower longevity, pollen fertility); 2) to determine the potential for self-compatibility in *N. phoenicea*; 3) to determine the effect of delayed style curvature on reproductive success; and 4) to determine whether prior pollination prevents style curving in this species. Following Ruan *et al.* (2010) for the Malveae tribe, we hypothesized that *Navaea phoenicea* shows a functional protandry system, in which outcrossing is promoted but reproductive success is assured. Experiments involving cross-pollination treatments, seed fitness, style manipulation, stigma receptivity, pollen/ovule ratio and observation of pollen tubes were performed. By addressing these issues, this study is the first to approach specific questions on the reproductive ecology of a Macaronesian palaeoendemic and provides further information on the evolution of delayed selfing in the Malvaceae family.

MATERIALS AND METHODS

Species and area of study

Navaea phoenicea is narrowly distributed in two populations across the northern slopes of the two oldest volcanic cores of Tenerife Island, Teno and Anaga mountain ranges, at altitudes ranging 400-750 meters in thermophilous or laurel forests. Flowering period starts with the first post-aestival rains and before new leaves grow in autumn, spanning occasionally until the end of February. Flowers are distributed either isolated or in terminal racemes of unknown longevity. Anther dehiscence, stigma receptivity, phenology and mating system are not known and are the subject of this paper. This study was carried out between 2005 and 2009 along three flowering periods in eight subpopulations (table 1).

Flower longevity, phenology and synchrony

We monitored 20 flower buds hourly in CHN population until corolla dehiscence for different parameters: i) proportion of open anthers; ii) proportion of anthers hosting pollen; iii) style longitude; iv) time of corolla closure and v) the angle for style curvature.

Phenology was monitored over three flowering periods with weekly visits to all subpopulations. In every visit number of flowers, ripe and unripe fruits were counted in each individual. Flowering synchrony between pairs of subpopulations was estimated by calculating the overlapping coefficient Δ_4 implemented in *overlap* package (Meredith & Ridout, 2013) in R software (R Core Team,

Table 2. Pairwise overlap coefficients Δ4 between subpopulations calculated for a 95% bootstrap CI. Bold numbers indicate significant differences in Δ4 coefficient (p<0.05).

	CAR	CEC	CHN	CVN	REP	TAB	TEN
CEC	**0.697**						
	0.705						
CHN	**0.684**	0.833					
	0.676	0.800					
CVN	**0.650**	0.790	0.864				
	0.756	0.858	0.856				
REP	**0.658**	0.795	0.846	0.888			
	0.744	0.849	0.844	0.873			
TAB	**0.633**	0.790	0.887	0.785	0.785		
	0.673	0.801	0.673	0.873	0.835		
TEN	**0.645**	0.842	0.879	0.817	0.858	0.894	
	0.673	0.782	0.882	0.882	0.835	0.872	
TOP	**0.645**	0.809	0.893	0.803	0.844	0.877	0.857
	0.682	0.782	0.882	0.882	0.839	0.87	0.885

2013). The density plot function implemented in the package was used to build flowering density diagrams to visualize flowering patterns and synchrony between subpopulations.

Self-compatibility

In each population we applied the following treatment to random buds in each plant prior opening: i) control (C), non-manipulated flowers; ii) autogamy (A), bagged flowers and no anther manipulation; iii) reinforced/supplemented autogamy (RA), emasculated flowers prior to anthesis and subsequently pollinated with stored self-pollen upon stylar branch exertion; iv) geitonogamy (G), emasculated flowers prior to anthesis and subsequently pollinated with pollen from the same individual; v) reinforced or supplemented xenogamy treatment (RX), emasculated flowers prior to anthesis and subsequently pollinated with outcrossing pollen after style had grown; and vi) apomixes (AP) emasculated and bagged. Treatments ii-vi included bagging with tulle boxes. We collected the fruits from flowers which successfully fructified. Seeds were weighted as a measure of fitness and sowed under greenhouse conditions after manual scarification to assess seed viability individually. Each treatment was applied to two flowers per individual in five to ten individuals, in eight subpopulations.

To evaluate the differences in number of viable seeds per fruit and seed fitness (i.e. seed weight) among treatments, we fitted generalized linear mixed models (GLMMs) with lme4 package (Bates *et al.*, 2013) implemented in R. Models included treatments, population and year as fixed factors and individual plants as random factor to control for potential individual variability in the response variables. We assumed a binomial error distribution with a logit link function for germinating seeds and an exponential error distribution for seed weights. Differences between treatment means were pairwise tested through multiple comparisons, using Bonferroni-corrected tests implemented in multcomp package (Hothorn *et al.*, 2008).

To detect pre-zygotic barriers, an additional subset of flowers was bagged, emasculated and further pollinated with an excess of either self or outcrossing pollen. Styles were cut 24 hours later and stored in a mix of 30% glycerine+70% ethanol. Styles were washed in the laboratory with NaOH and stained with Aniline blue following Kearns & Inouye (1993) for manually count.

Pollen longevity

Pollen viability was assessed by testing presence of peroxidase activity. A set of 15 floral buds per four populations (TAB, CHN, CAR, TEN) were randomly selected and bagged prior to anthesis. Four samples of 20 pollen grains were extracted every 12 hours and placed in a 0.5 µl Eppendorf tube with distilled water and the time to open the tube due to oxygen pressure was measured and taken as an estimate of the intensity of the peroxidase activity. In order to compare *in vitro* and natural conditions, 60 pollen grains were collected from flowers immediately after anthesis and placed in darkness at 12° in a petri dish containing a 15% sucrose solution following Kearns & Inouye (1993). We counted number of germinated pollen grains 12 hours after sowing. This procedure was repeated every 12 hours until corolla closure.

Stigma receptivity and style curvature

Stigma receptivity was assessed by checking peroxidase activity with Peroxtesmo paper test (manuf. Macherey-Nagel) every 12 hours since stigma growth in five random bagged buds in four populations (CHN, TAB, TEN and CEC). From an additional subset of buds, we cross-pollinated flowers at early style growth and with styles curved to approximately 180°, and then fixed and stained stigmas as described above for manually counting of pollen grains. Here, 15 buds per treatment in five plants in two populations (TAB and TEN) were randomly chosen. We applied each treatment to three stigmas per flower.

Table 3. Results of the generalized linear mixed model for the effect of the pollination treatment, year, population and plant, as random factor, on the reproductive response variables a) Seed weight and b) Number of viable seeds per fruit (n=300).

	a) Seed weight		b) Number of viable seeds	
Source of variation	**F**	**P-value**	**F**	**P-value**
Treatment	89.68	<0.0001	9.89	<0.0001
Population	16.85	0.022	0.3324	0.097
YearW	11.62	0.650	0.56	0.016
Plant	16.73	0.13	1.80	0.487

Table 4. Percentages of stigmas showing peroxidase activity. Time is presented as 12 hours time slots starting at the beginning of style exertion.

Time slot	Percentage
1-8	100%
9	62.5%
10 (corolla closure)	12.5%

To check if early pollination precluded the style curvature, we cross-pollinated bagged flowers 12 hours after stigma growth. 48 hours after pollination, stylar branches were cut and the degree of style curvature was estimated by means of trigonometric calculations depending of style length, curvature at the stigma and distance to the staminal column. We applied each treatment to a set of 20 emasculated and bagged buds in CHN population. Approximately half of the styles were pollinated in each flower and the rest left as control group.

Pollen/ovule ratio

To account for pollen/ovule ratios (P/O, Cruden, 1976) 20 flowers were randomly collected in 2006 in ten populations before the opening of the corolla. Anthers were manually opened and pollen grains counted with a magnifier together with the number of ovules. P/O was considered as the average number of pollen grains divided by the average number of ovules.

RESULTS

Flower longevity and phenology

The average lifespan of the flower ranged between six days and six days and 12 hours. Anthesis always took place in less than 12 hours after flower opening. At the beginning of the style exertion (60 hours) more than 80% of pollen in anthers had been already shed (figure 2). Although several flowers maintained anthers with pollen by the moment of the exertion (timeslot 5), 100% of pollen in all the flowers was shed at the moment that the average angle of style curvature was greater than 90° (timeslot 10, figure 2). This indicated a strong temporal separation between male and female functions.

Flowering period ranged from September up to February (figure 3). Overlap coefficients showed consistent high values across pairwise comparisons except for CAR population (table 2). In this population, confidence intervals for overlap values were significantly lower with respect to the rest of populations, denoting a shift in flowering time. Figure 2 indicates hence an earlier bloom in Carboneras population than the rest of populations. Interestingly, the density plots allowed to detect in all populations a frequent pattern consisting of two flowering peaks.

Self-compatibility

Both seed weight and number of viable seeds per fruit varied significantly among treatments but not among plants, years or populations (table 3a). Outcrossing, selfing and geitonogamy treatments yielded higher seed weights than no supplemented autogamy and control group treatments (figure 4a).

Similar results were obtained for the number of viable seeds per treatment (table 3a). We found no significant differences between supplemented treatments, which were significantly higher values for seed numbers than control and non-supplemented self-fertilization. The three treatments supplemented and non-supplemented autogamy and geitonogamy produced viable seeds (figure 4b).

We detected a high proportion of pollen grains that germinated on the stigmas producing pollen tubes (85.09 ±3.79 %). There were no significant differences between self and outcrossing pollen (n=808, t=-0.279, p=0.7803).

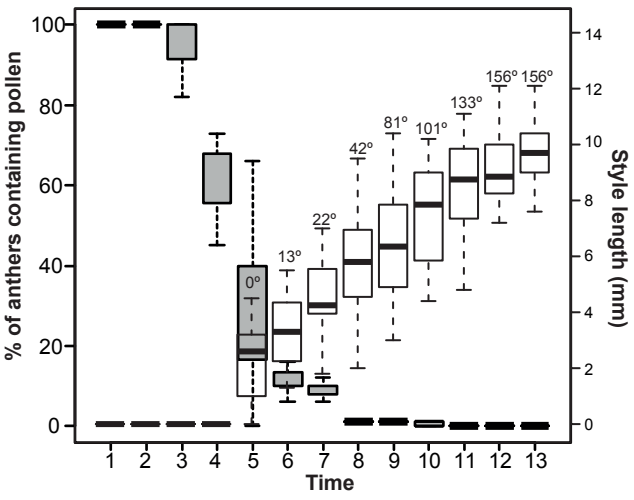


Figure 2. Percentages of anthers containing pollen (grey boxplots) and style length (white boxplots) measured across 12 hours intervals (6.5 days in total). Above boxplots for style length, numbers indicate the average style curvature of the measured flowers in degrees. 0 degrees indicate no curvature, whereas 180º indicate a complete curvature downwards.

Table 5. One factor ANOVA results for pollination of styles to test style curvature pollination (n=180)

Percentage of germinated pollen grains			
Recently exerted styles		Curved styles	
82.27±11.27		84.53±11.70	
ANOVA test			
Source of variation	df	F	P-value
Style curvature	1	1.647	0.2009
Population	1	0.315	0.576

Pollen longevity

Peroxidase activity decreased over time (df=1, F=3604, $p=2.2*10^{-16}$) with an initial time of 15.40 minutes (SD=1.15) at the anthesis stage up to 24.27 minutes (SD=1.24) at the last measured stage. Peroxidase activity was nevertheless present across all time periods, but tended to decrease over time. In vitro germination test showed most of the pollen was able to germinate across the whole length of the flowering period, although the proportion of germinated pollen grains significantly decreased over time (n=778, F=3283, $p=2.2*10^{-6}$). At the anthesis stage, 85.29% of pollen grains germinated, while at the flower closure, 65.50% of pollen grains germinated.

Stigma receptivity and style curvature

100% of stigmas showed peroxidase activity at all the stages except at flower closure stages (62.5% to 12% of flowers, table 4). Hence, since the opening of the flower, stigmas were receptive even before exertion.

Pollination of recently exerted and curved at almost 180° stigmas did not show significant differences in the percentage of pollen tubes grown (table 5), with high rates of germination in both cases (82.27% and 84.53% for recently exerted and curved styles, respectively).

Finally, experiment for style curvature prevention upon pollination did not show significant differences between pollinated and non-pollinated styles (table 6). All the styles reached curvatures near 160° (i.e., curved down towards staminal column). In the comparison between stigmatic development stages, we found similar percentages of pollen tubes grown as when analyzing pollen longevity (83.40% ± 11.82).

Table 6. One factor ANOVA results for pollination of styles to test style curvature pollination (n=408)

Degree of curvature (degrees)	Non-pollinated	Pollinated
	160.33±16.55	157.90±17.30
Source of variation	F	P-value
treatment	2.101	0.1479

Pollen/ovule ratio

N. phoenicea flowers had a mean of 21.84 ovules and 111 anthers per flower. The mean of total pollen grains per flower was 11.107. Each anther showed on mean 99.29 pollen grains. P/O ratio calculated was then 504.66 (table 7).

DISCUSSION

The combination of experiments performed in this work allows to detect the limitations of delayed selfing mechanism in *Navaea phoenicea*. Our results indicate no temporal segregation of pollen viability or stigma receptivity that can preclude self-pollination. There is instead a temporal separation of male and female flower stages due to an early pollen shed, which occurs even in the absence of floral visitors. Hence it should be considered as an outcrossing species rather than a facultative autogamous species, despite the lack of SI mechanisms.

Flower longevity and phenology.

Flower lifespan was at least of six days with two differentiated male and female stages, with little overlapping between them, as no pollen remained in the anthers under natural conditions for delayed selfing opportunities. Individual flowers were surveyed under natural conditions and thus allowing flower visitors to remove pollen. However, even in the bagged flowers for autogamy treatments, the same pattern of early shedding of pollen was detected. The observed temporal segregation of male and female functions is clear as for a dichogamic species, and also raises another issue: as the male stage is shorter than the female one, this fact brings two questions. The first one is how this may affect the reproductive success of this species considering that self-pollination is not functional. The window opportunity for pollen dispersal is limited to a 24 hours period only. The second one is the allocation of resources to each sex, specially to the short male period. Because pollinators should be removing as much pollen as possible in such short period, the allocation of reproductive resources to achieve an optimal male fitness should be high, or saturating (Brunet, 1992).

The measured flowering period was long, lasting along the whole autumn period and entering well into the winter season. Flowering period was weakly overlapped with

a spatially coincident ornithophilous species, *Canarina canariensis*, a frequent species in the thermophilous shrubland, which starts blooming at the beginning of January. On the other hand, there are eventual coincident with *Isoplexis canariensis* (Scrophulariaceae) which may flower until September (Rodríguez-Rodríguez & Valido, 2008) although the occurrence within the same habitats is not so frequent. Whilst *N. phoenicea* grows predominantly, but not exclusively in the thermophilous vegetation, *I. canariensis* is associated to the laurel forest.

The overlapping coefficients obtained showed synchronous flowering across all the sampled years. To our knowledge, the overlap algorithm has not been applied before to explore flowering phenology patterns. This approach provided a useful, straightforward method that allowed detecting key results: it detected that Carboneras subpopulation flowered ahead of the rest of populations along the three years surveyed. Climatic determinants may be invoked to explain phenology patterns. For instance, *N. phoenicea* appears to be dependent of climate constraints for leaf and flower emergence. Leaves are lost in June upon the arrival of the dry season, not because of day light extent but because of drought (personal observation), and new leaf emergence occurs in the late autumn after flowering period has started. On the other hand, blooming occurs after the first autumn rains. In chapter 1, we were able to extract climate data for the ensemble of populations. These data allows detecting some differences among the climate conditions of the populations. Carboneras subpopulation shows the lowest evapotranspiration rate, suggesting a

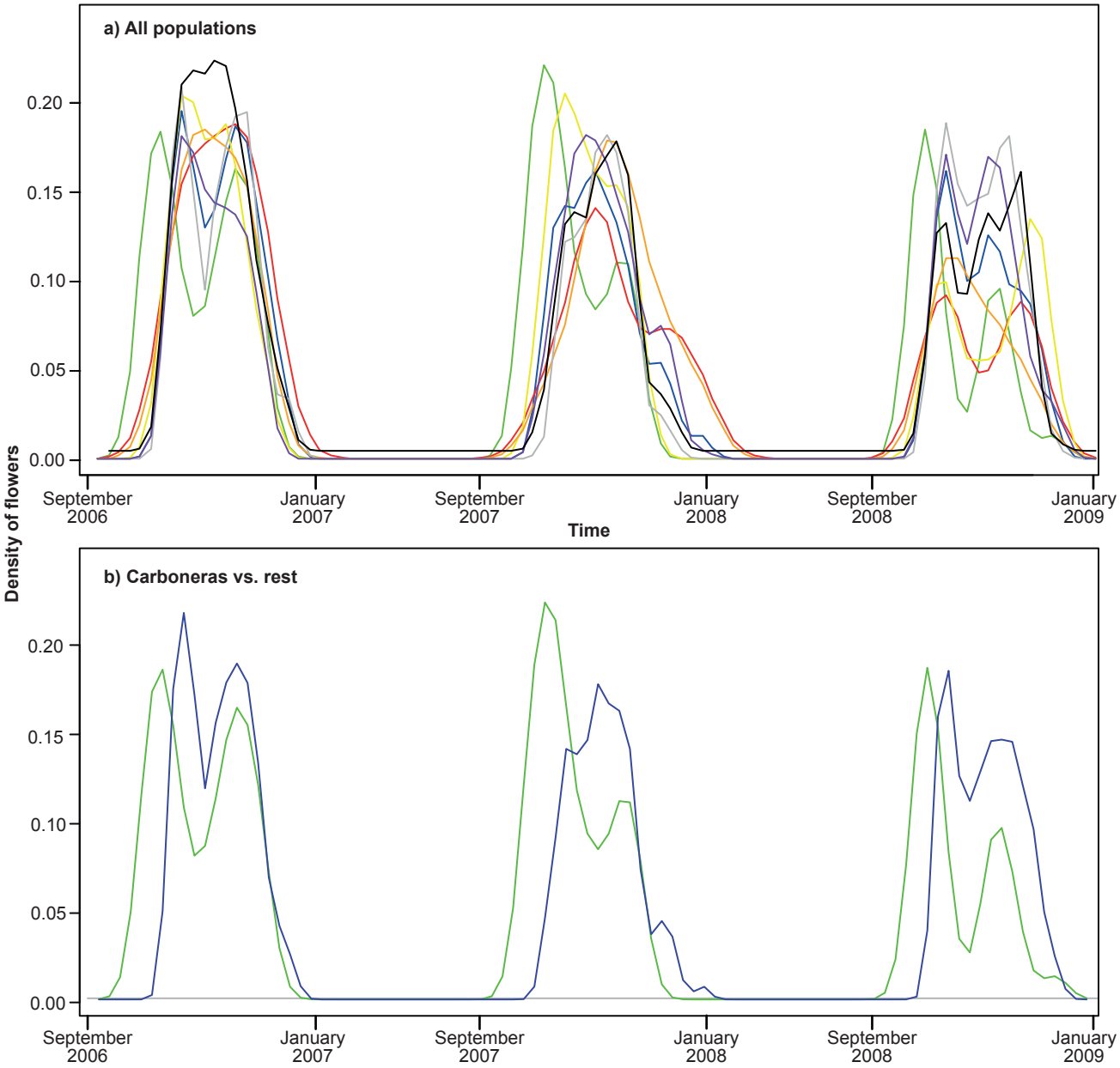


Figure 3. Kernel density diagrams displayed for a) all the populations; and b) Carboneras population versus all the rest of populations.

Table 7. Pollen ovule ratio (P/O) parameters for ***Navaea phonicea***

	Mean	SD
Mean number of ovules per flower	21.84	5,62532621
Mean number of Anthers per flower	111	26,9167413
logP/O	2,70	
P/O ratio	504.66	
Mean polen per anther	99.29	14,4354275
Total pollen per flower	11107	2285,79372

better water balance permitting flowering in advance. This can result in a slight temporal reproductive isolation despite no isolation by distance. In chapter 2, Carboneras was identified as the least admixtured subpopulation in the Anaga massif. Second, the density plots detected that two flowering peaks within one flowering period was generalized and occurred synchronously among populations. This quick response in bloom intensity may be attributed to resource allocation, particularly water as flowering period appears to be dependent on water balance.

Another two key elements arise from the phenological survey carried out. One is that flowering period was slightly shorter in the last monitored period (2008-2009). The second one is the remarkable pattern of two blooming peaks that recurrently appear across periods in most of the populations. Again, the hypothesis on resource allocation and response to climate conditions should be explored.

The efficiency of self-compatibility

Hand pollination experiments showed a total self-compatibility in the studied species and strong pollen limitation for delayed self-fertilization. The absence of differences in pollen tube growth or seed sets allow to discard the effects of inbreeding depression. Numbers of viable seeds or seed weight were neither influenced by pollen origin. However, non-supplemented self-pollination yielded lower seed sets and hence lower seed weights, showing that delayed selfing in the nature is unsuccessful due to pollen limitation. The treatment accounting for autogamy (A) allowed determining the ratio of success of delayed selfing. Experiments on pollen and stigma fertility show that for the whole flower lifespain male and female function could overlap. The factor explaining a low performance of the delayed selfing mechanism in this species appears then to be the lack of overlap between male and female stages. As shown by the monitoring of flower stages, pollen is shed well before self-pollination can occur. Even though if flowers in this treatment were bagged, pollen already remained scarce at the start of the female stage driving a poor performance for the delayed

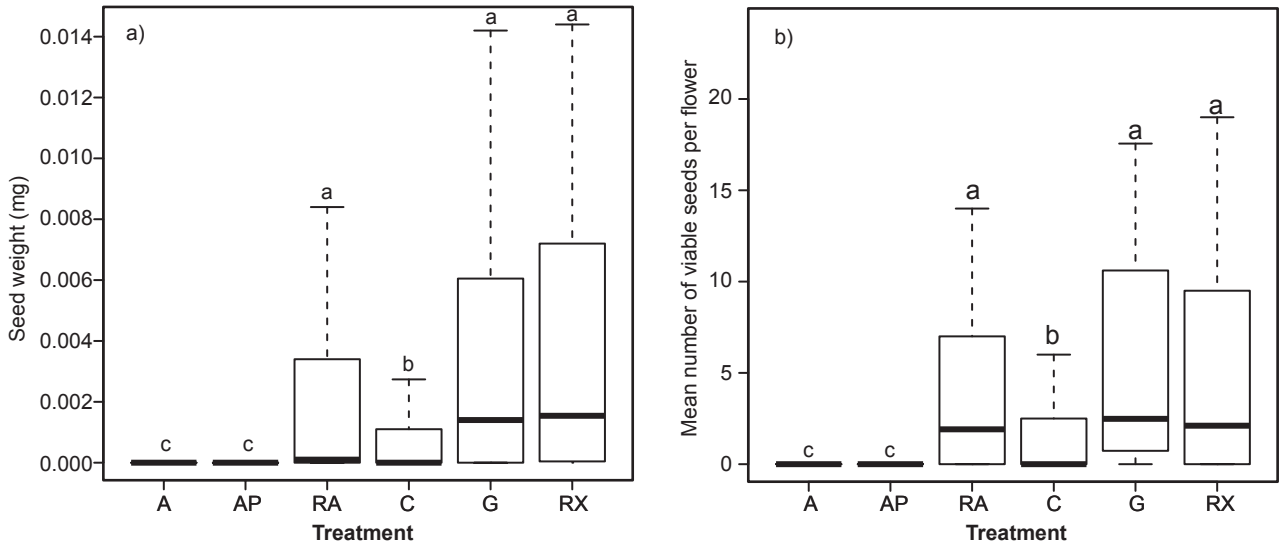


Figure 4. a) Boxplots for individual seed weights (grams) by flower experimental treatment; b) Number of viable seeds by flower experimental treatment. Letters above boxplot indicate significantly different groups.

selfing mechanism. These findings are consistent with the P/O values determined for the species. Pollen/ovule ratio situated *N. phoenicea* within the range of ratios reported for the Malvaceae family, therefore consistently considered between Facultative Xenogamous or Xenogamous under the categories proposed by Cruden (2000). It is worth noting that, in contrast to other species (Ruan *et al.*, 2010), stylar branches movements were not facultative and an excess of supplemented pollen did not prevent style curvature, which continued to curve towards the staminal column.

Out of the Malvaceae lineage, the failure of delayed self-fertilization has been reported for *Kalmia latifolia* (Ericaceae, Rathcke, 2003). Within the family, delayed selfing has been reported for *Hibiscus trionum* (Ramsey *et al.*, 2003; Seed *et al.*, 2006), *H. laevis* (Klips & Snow, 1997) and *Kosteletzkya virginica*, (Ruan *et al.*, 2008) and inability for self-pollination has been reported for *H. moscheutos* (Spira *et al.*, 1992). More recently, the survey by Ruan *et al.* (2010) across the whole family reported style curvature in three out of seven species from the more closer Malveae tribe, but did not provide any assessment of its effectiveness, except for *Althea rosea*. In this review authors differentiated between two roles for style curvature in the family. Type I occurs among the majority of tribes and style curvature occurs in the presence of viable pollen, whereas type II occurs in the Malveae tribe. Here, style curves towards staminal column after pollen is shed, this is, under lower pollen availability. Therefore, the results obtained for *Navaea phoenicea* are in line with the Malveae style response type II although pollen viability remained higher in *N. phoenicea* than in other Malveae species. This provides evidence that style curvature in this species may respond to male-female avoidance or maximization of outcrossing rather than to reproductive assurance. A second evidence for this is the absence of control of stylar movements, since curvature was not stopped by pollination. To our knowledge experiments have been carried out in the Malvaceae for *Hibiscus*, but not for the Malveae tribe. In *Hibiscus* the style curvature appears to be facultative regarding the amount of pollen previously received and it is related to delayed selfing. In *N. phoenicea*, an excess of pollen did not preclude style curvature, which occurs invariably and cannot be considered then as facultative. Therefore results suggest as well that stylar movement may be associated with outcrossing promotion instead of delayed selfing.

From the perspective of the evolution of mating systems in islands, *N. phoenicea* does not appear to be in line with the predicted prevalence of self-compatible species. The ecological advantages conferred by selfing to early island colonizers are lost for this taxon, but self-fertilization may not be equally adaptive for a colonizer and a relict species. Instead, the adaptation to complete outcrossing may increase gene flow against gene drift and founder effects. This is especially relevant concerning *N. phoenicea*. As an endangered species, both inbreeding depression and failure in reproductive ability may be reasons for concern. For the first factor of risk, the species conversely shows greater genetic diversity than expected (chapter 2), due to high gene flow mediated by passerine pollinators. In general, outcrossing in the Canarian taxa leads to higher levels of genetic diversity (Pérez de Paz & Caujapé-Castells, 2013). Regarding the second factor, the reproductive assurance relies on the role of the opportunistic generalist passerines which pollination is unequal between current visitors, while the original pollinators may not be present any more. If in the past the species benefited from high reproductive success due to efficient pollinators, then losing delayed selfing – either to maximize outcrossing or male and female functions - may be adaptive. Since this is can be contemplated as an specialization, it may be at odds with the fact that most commonly, autonomous selfing is related to flower specialization (Fenster *et al.*, 2007). Further work is nevertheless needed to map the evolution and the role of delayed selfing across the Malveae tribe.

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These results provided evidence for alternative meanings for the forms of approach herkogamy or protandry in the Malvaceae family, and contribute to extend our knowledge about the role of stylar movement and the evolution of mating strategies across the family. Rates of self-fertilization and the display of floral traits for optimizing the efficiency of flower visitors have determinant implications for conservation and spatial genetic arrangements of populations, which need to be studied together with important reproductive features such as temporary flower synchrony or pollinator availability. For example, plant-pollinator interactions and pollinator behavior as determinants for selfing rates have received little attention in the literature (Devaux *et al.*, 2014). Given the evolutionary distinctiveness that the reproductive biology confers to *N. phoenicea* and the rest of the Macaronesian Bird-Flower element, the species provides opportunities to test the pollinator efficiency of current flower visitors and their contribution to outcrossing.

REFERENCES

Anderson G.J., Bernardello G., Stuessy T.F., & Crawford D.J. (2001) Breeding system and pollination of selected plants endemic to Juan Fernandez Islands. *American Journal of Botany*, **88**, 220–233.

Auld J.R. & Rubio de Casas R. (2013) The correlated evolution of dispersal and mating-system traits. *Evolutionary Biology*, **40**, 185–193.

Baker H.G. (1955) Self-Compatibility and Establishment After "Long-Distance" Dispersal. *Evolution*, **9**, 347–349.

Barrett S.C.H. (2004) Flower diversity and plant mating strategies. *McGraw-Hill Yearbook of Science & Technology* pp. 110–113. McGraw-Hill, New York.

Barrett S.C.H. (2010) Why reproductive systems matter for the invasion biology of plants. *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (ed. by D.M. Richardson), pp. 195–210. Blackwell Publishing Ltd, Oxford.

Barrett S.C.H., Richards A.J., Bayliss M.W., Charlesworth D., & Abbott R.J. (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **358**, 991–1004.

Barringer B.C. (2007) Polyploidy and self-fertilization in flowering plants. *American Journal of Botany*, **94**, 1527–33.

Bates D., Maechler M., & Bolker B. (2013) lme4: Linear mixed-effects models using S4 classes. Version 0.9.

Bertin R.I. & Newman C.M. (1993) Dichogamy in angiosperms. *Botanical Review*, **59**, 112–152.

Brunet J. (1992) Sex allocation in hermaphroditic plants. *Trends in Ecology & Evolution*, **7**, 79–84.

Busch J.W. & Delph L.F. (2012) The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Annals of Botany*, **109**, 553–562.

Carlquist S. (1966) The biota of long-distance dispersal I. Principles of dispersal and evolution. *The Quarterly review of biology*, **41**, 247–270.

Castro-Urgal R. & Traveset A. (2014) Differences in flower visitation networks between an oceanic and a continental island. *Botanical Journal of the Linnean Society*, **174**, 478–488.

Cheptou P.O. (2012) Clarifying Baker's law. *Annals of Botany*, **109**, 633–641.

Crawford D.J., Archibald J.K., Stoermer D., Mort M.E., Kelly J.K., & Santos-Guerra A. (2008) A Test of Baker's Law: Breeding Systems and the Radiation of *Tolpis* (Asteraceae) in the Canary Islands. *International Journal of Plant Sciences*, **169**, 782–791.

Cruden R.W. (1976) Intraspecific Variation in Pollen-Ovule Ratios and Nectar Secretion - Preliminary Evidence of Ecotypic Adaptation. *Annals of the Missouri Botanical Garden*, **63**, 277–289.

Cruden R.W. (2000) Pollen grains: Why so many? *Plant Systematics and Evolution*, **222**, 143–165.

Devaux C., Lepers C., & Porcher E. (2014) Constraints imposed by pollinator behaviour on the ecology and evolution of plant mating systems. *Journal of Evolutionary Biology*, **27**, 1413–1430.

Eckert C.G. (2000) Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology*, **81**, 532–542.

Eckert C.G., Kalisz S., Geber M.A.. M. a, Sargent R., Elle

E.E.., Cheptou P.O., Goodwillie C., Johnston M.O.. M.O., Kelly J.K.J.K.., Moeller D.A., Porcher E.. E., Ree R.H.. R.H., Vallejo-Marín M., & Winn A.A.A.. (2010) Plant mating systems in a changing world. *Trends in ecology & evolution*, **25**, 35–43.

Escobar García P., Schönswetter P., Fuertes Aguilar J., Nieto Feliner G., & Schneeweiss G.M. (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). *Molecular Phylogenetics and Evolution*, **50**, 226–39.

Fenster C.B., Marte S., & Marten-Rodriguez S. (2007) Reproductive assurance and the evolution of pollination specialization. *International Journal of Plant Sciences*, **168**, 215–228.

Ferrer M.M. & Good S. V (2012) Self-sterility in flowering plants: preventing self-fertilization increases family diversification rates. *Annals of Botany*, **110**, 535–553.

Fowler N.L. & Levin D.A. (1984) Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist*, **124**, 703.

Gibbs P. (2014) Late-acting self-incompatibility–the pariah breeding system in flowering plants. *New Phytologist*, **203**, 717–734.

Goodwillie C., Kalisz S., & Eckert C.G. (2005) The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 47–79.

Gustafsson Å. (1948) Polyploidy, lifeform and vegetative reproduction. *Hereditas*, 1–22.

Herlihy C.R. & Eckert C.G. (2002) Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature*, **416**, 320–323.

Hothorn T., Bretz F., & Westfall P. (2008) Simultaneous inference in general parametric models. *Biometrical journal*, **50**, 346–363.

Igic B., Lande R., & Kohn J.R. (2008) Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences*, **169**, 93–104.

Kalisz S., Vogler D.W., & Hanley K.M. (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature*, **430**, 884–887.

Karron J.D., Ivey C.T., Mitchell R.J., Whitehead M.R., Peakall R., & Case A.L. (2012) New perspectives on the evolution of plant mating systems. *Annals of Botany*, **109**, 493–503.

Kearns C.A. & Inouye D.W. (1993) *Techniques for Pollination Biologists*. University Press of Colorado, Colorado.

Klips R.A. & Snow A.A. (1997) Delayed autonomous self-pollination in Hibiscus laevis (Malvaceae). *American Journal of Botany*, **84**, 48–53.

Lloyd D.G. (1992) Self- and Cross-Fertilization in Plants. II. The Selection of Self- Fertilization. *International Journal of Plant Sciences*, **153**, 370.

Meredith M. & Ridout M. (2013) Overlap: Estimates of coefficient of overlapping for animal activity patterns. Version 0.24.

Moreno-Saiz J.C. (2008) *Lista Roja de Flora Vascular Española*. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid.

Olesen J.M. & Valido A. (2004) Lizards and birds as generalized pollinators and seed dispersers of island plants. *Insular Ecology* (ed. by J.M. Fernández Palacios and C. Morici), pp. 229–250. Asociación Española de Ecología Terrestre y Excmo.Cabildo Insular de La Palma, La Palma.

Pannell J.R. (2006) Effects of colonization and metapopulation dynamics on the evolution of plant sexual systems. *Ecology and evolution of flowers* (ed. by L.D. Harder and S.C.H. Barrett), pp. 223–238. Oxford University Press, Oxford.

Pannell J.R. (2015) Evolution of the mating system in colonizing plants. *Molecular Ecology*, **24**, 2018–2037.

Pannell J.R., Auld J.R., Brandvain Y., Burd M., Busch J.W., Cheptou P.O., Conner J.K., Goldberg E.E., Grant A., Grossenbacher D.L., Hovick S.M., Igic B., Kalisz S., Petanidou T., Randle A.M., Casas R.R. De, Pauw A., & Vamosi J.C. (2015) The scope of Baker's law. *New Phytologist*, **208**, 656–667.

Pérez de Paz J. & Caujapé-Castells J. (2013) A review of the allozyme data set for the Canarian endemic flora: causes of the high genetic diversity levels and implications for conservation. *Annals of Botany*, **111**, 1059–73.

R Core Team (2013) R: A Language and Environment for Statistical Computing. Version 3.1.2.

Ramsey J. & Schemske D.W. (1998) Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics*, **29**, 467–501.

Ramsey M., Seed L., & Vaughton G. (2003) Delayed selfing and low levels of inbreeding depression in *Hibiscus trionum*

(Malvaceae). *Australian Journal of Botany*, **51**, 275–281.

Rathcke B.J. (2003) Floral longevity and reproductive assurance: Seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *American Journal of Botany*, **90**, 1328–1332.

Rodríguez-Rodríguez M.C. & Valido A. (2008) Opportunistic nectar-feeding birds are effective pollinators of bird-flowers from Canary Islands: Experimental evidence from *Isoplexis canariensis* (Scrophulariaceae). *American Journal of Botany*, **95**, 1408–1415.

Ruan C.-J.J., Chen S.-C.C., Li Q., & Teixeira da Silva J. a. (2011) Adaptive evolution of context-dependent style curvature in some species of the Malvaceae: A molecular phylogenetic approach. *Plant Systematics and Evolution*, **297**, 57–74.

Ruan C.-J.J., da Silva J.A.T.., Qin P.P.., & Teixeira da Silva J. a. (2010) Style curvature and its adaptive significance in the Malvaceae. *Plant Systematics and Evolution*, **288**, 13–23.

Ruan C.-J.J., Zhou L.J., Zeng F.Y., Han R.M., Qin Q., Lutts S., Saad L., & Mahy G. (2008) Contribution of delayed autonomous selfing to reproductive success in *Kosteletzkya virginica*. *Belgian Journal of Botany*, **141**, 3–13.

Seed L., Vaughton G., & Ramsey M. (2006) Delayed autonomous selfing and inbreeding depression in the Australian annual *Hibiscus trionum* var. *vesicarius* (Malvaceae). *Australian Journal of Botany*, **54**, 27.

Spira T.P., Snow A.A., Whigham D.F., & Leak J. (1992) Flower Visitation , Pollen Deposition , and Pollen-Tube Competition in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany*, **79**, 428–433.

Stebbins G.L. (1940) The significance of polyploidy in plant evolution. *The American Naturalist*, **74**, 54–66.

Stebbins G.L. (1950) *Variation and evolution in plants.* Columbia University Press, New York.

Stebbins G.L. (1957) Self fertilization and population variability in the higher plants. *The American Naturalist*, **91**, 337.

Trøjelsgaard K., Báez M., Espadaler X., Nogales M., Oromí P., Roche F. La, & Olesen J.M. (2013) Island biogeography of mutualistic interaction networks. *Journal of Biogeography*, **40**, 2020–2031.

Tsitrone A., Duperron S., & David P. (2003) Delayed selfing as an optimal mating strategy in preferentially outcrossing species: Theoretical analysis of the optimal age at first reproduction in relation to mate availability. *The American Naturalist*, **162**, 318–331.

Valido A., Dupont Y.L., & Olesen J.M. (2004) Bird-flower interactions in the Macaronesian islands. *Journal of Biogeography*, **31**, 1945–1953.

Wright S.I., Ness R.W., Foxe J.P., & Barrett S.C.H. (2008) Genomic Consequences of outcrossing and selfing in plants. *International Journal of Plant Sciences*, **169**, 105–118.

4 Differentiated pollination efficiency of generalist passerines on the Macaronesian palaeoendemic *Navaea phoenicea* (Malvaceae)

Eficiencia de la polinización diferenciada de los paseriformes generalistas sobre el paleoendemismo Macaronésico *Navaea phoenicea* (Malvaceae)

4. Differentiated pollination effectiveness of generalist passerines on the reproductive success of the Macaronesian palaeoendemic Navaea phoenicea (Malvaceae)

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ABSTRACT

The oceanic archipelago of the Canary Islands has observed the convergent adaptation of independent plant lineages with a bird-pollination syndrome, in the absence of specialist nectarivorous birds. The evolutionary scenarios that have allowed the apparition of bird pollinated flowers may be different for each lineage, with cases with an ancient origin and others with a more recent one. For several species the efficiency of current visitors is not known. We studied the quantitative and qualitative components of pollination efficiency in an endemic mallow to Tenerife Island, *Navaea phoenicea*. We assessed visitation frequency patterns of birds and insects on the one hand, and the efficiency of each species with respect to male and female functions on the other. Birds showed very differentiated components, with two warbler species showing high quality components but low visit frequency, and Canarian chiffchaffs and blue tits showing higher frequencies but lower efficiency, which were near 0 in the case of the blue tits. At the same time we found remarkable frequencies of insect visits, which acted as nectar robbers. Pollination efficiency of three of the four bird species may be high enough to maintain a selective pressure on floral traits, although the relict origin remains more plausible. In particular, the behaviour of birds plays a crucial role on the fructification success, as perching habit, shared by part of the generalist passerines and warblers, is fairly more efficient. This feature, along with the detailed examination of floral traits can contribute to disentangle the origin of ornithophily in *N. phoenicea.*

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INTRODUCTION

The isolated condition of oceanic islands has hindered colonisation and dispersal processes and has driven in many instances a disharmonic and impoverished assemblage of the biota. One of the consequences is that plant-pollinator interactions and the networks they constitute are more depauperated than those in the continents (Trøjelsgaard & Olesen, 2013; Castro-Urgal & Traveset, 2014), present super-generalist species (Olesen *et al.*, 2002) and favour new plant-pollinator interactions (Traveset *et al.*, 2015). These new interactions have arisen from the higher abundance of animal groups by density compensation which results in intraspecific competition and therefore in trophic niche expansion.

The occurrence of bird-flower interactions occurs particularly in the tropical realms, where specific mutualistic pollination interaction often occurs between flowers and nectar feeding specialist birds (Anderson, 2003). However, generalist passerines have been also reported in temperate areas occasionally visiting flowers in the search of nectar, and the incorporation of nectar to diet by generalist passerines may aim for diet completion when food sources are scarcer. This phenomenon has been reported to be fairly more frequent in islands than in mainland areas (Valido *et al.*, 2004), and it has been interpreted as the response of birds to trophic niche expansion and density compensation (Olesen & Valido, 2003). Following a similar response, lizard species have been observed expanding their trophic niche and incorporating frugivory and nectarivory dietary patterns (Valido & Nogales, 2003; Nogales *et al.*, 2012; Trøjelsgaard *et al.*, 2013).

Among the examples of islands where visits by generalist passerines birds have been observed, the Macaronesian archipelagos have been a subject of scientific interest the last decide (Ojeda, 2013). The Macaronesian region is composed by five oceanic archipelagos in the Atlantic. At least 16 endemic plant taxa have been reported to be frequently visited by generalist passerines within the genus *Canarina* and *Musschia* (Campanulaceae), *Isoplexis* and *Scrophularia* (Scrophulariaceae), *Echium* (Boraginaceae), *Lotus* (Leguminosae) and *Navaea* (Malvaceae). The passerines foraging in the potential ornithophilous flowers are the Canarian blue tit *Cyanistes teneriffae*, the Canarian chiffchaff *Phylloscopus canariensis*, the chaffinch *Fringilla coelebs* (Fringillidae), the Canary bird *Serinus canarius* and three species of warblers: Blackcap (*Sylvia atricappilla*), Sardinian (*S. melanocephala*) and Spectacled (*S. conspicillata*). The assemble of these taxa is known as the "Macaronesian Bird Flower Element" (MBE), and it shares common floral traits that appear to be adapted to bird-pollination, such as diluted nectar, red conspicuous corollas, etc. (Faegri & Van Der Pijl, 1979; Waser *et al.*, 1996; Cronk & Ojeda, 2008). Hence, they have been considered to show a bird pollination syndrome that have evolved separately in each plant lineage (Olesen, 1985). After the initial expositions of the phenomenon (Vogel *et al.*, 1984; Olesen, 1985), Valido *et al.* (2004) proposed two hypotheses to explain the evolution of this insular syndrome. Based on the origin of each putative ornithophilous lineage, authors differentiated between a recent origin (*de novo* hypotheses) for *Echium* as result of a recent radiation and an ancestral African origin for *Navea phoenicea* and *Canarina canariensis*. Sister *Canarina* species in continental Africa are indeed pollinated by specialist sunbirds and are relict species (Olesen *et al.*, 2012). These taxa are considered relict species related to past African flora, and ornithophily may have evolved in the past in the continent in response to sunbirds. Although the major drawback in this hypothesis is the lack of bird fossils found in Macaronesia the existence of extinct generalist remains plausible (Valido *et al.*, 2004). For instance, there are rest of hummingbirds in Europe during the Eocene (Mayr, 2004) with evidence for bird-flower interactions at the same period (Mayr & Wilde, 2014).

After the proposal by Valido *et al.* (2004) to explain the syndrome, several studies have been conducted to explore ornithophilous floral traits on one hand, while others have deepened into individual species reproductive biology in order to validate both hypotheses. For *Isoplexis canariensis* and *Canarina canariensis* the effectiveness of birds as pollinators (namely chiffchaffs) was confirmed (Rodríguez-Rodríguez & Valido, 2008, 2011; Rodríguez-Rodríguez *et al.*, 2013, 2015). Conversely, the molecular phylogeny for the genus *Lotus* reveals a recent origin for the four Macaronesian species visited by birds (Ojeda *et al.*, 2013), but actual the evidence of the effectiveness of birds as pollinators remain absent. Recent several studies have extended the bird-flower mutualism to species in the temperate continents. For instance, *Anagyris latifolia* in the Canary Islands and *A. foetida* (Fabaceae), in the warmer areas of the Iberian Peninsula have been described as ornithophilous relict species based on floral traits and visitor efficiency. The interpretation of ornithophily here

has been first envisioned as a relict mutualism (Ortega-Olivencia *et al.*, 2005; Valtueña *et al.*, 2008) and afterwards as a derived character in both species (Ortega-Olivencia & Catalán, 2009). Likewise, three European *Scrophularia* species have been recently reported to have a mixed bird-insect mating system together with the Macaronesian *S. calliantha* (Ortega-Olivencia *et al.*, 2012). For these taxa the phylogenetic reconstruction of pollination systems supports a recent origin of vertebrate pollination from insect pollination which therefore provides evidence for the *de novo* hypotheses of *S. calliantha* (Navarro-Pérez *et al.*, 2013). The occurrence of interactions between generalist passerines in the European continent is nevertheless a frequent observed phenomenon which occurs mainly in food scarcity seasons, although the ecological implications still needs a great amount of research work (da Silva *et al.*, 2014).

In this paper we studied the role of generalist passerines in *Navaea phoenicea* to provide insights on the evolution of the bird pollination syndrome in Macaronesia. The species is endemic from Tenerife Island in the Canarian archipelago. From a phylogenetic perspective the species is a well-considered palaeoendemic taxon which is basal to the Malveae tribe lineage (Escobar García *et al.*, 2009). Unlike other taxa, the role of floral visitors and floral traits have been only superficially analysed in studies accounting for the ensemble of the MBE (Dupont *et al.*, 2004; Valido *et al.*, 2004; Ojeda, 2013). However, previous works on the mating system show that *Navaea phoenicea* is self-compatible but reproductive success relies essentially in outcrossing, as the delayed self-fertilization mechanism is not functional (chapter 3). Preliminary field observations have reported not only bird visits but also several insect species (Portellano & Fuertes, unpublished results). Similar patterns of bird visits along several insects have been reported for other species of the MBE (Rodríguez-Rodríguez & Valido, 2008; Ollerton *et al.*, 2009; Rodríguez-Rodríguez *et al.*, 2013). The relict condition of the species places the evolution of the bird pollination syndrome under the *de novo* hipótesis, but the phylogenetic isolation of the plant hinders the evolutionary interpretation of the development of adaptations to bird pollination. Within the related Malveae tribe, *Lavatera acerifolia*, which is endemic to the Canarian archipelago as well, has a Mediterranean origin and is the sister taxon of *Lavatera maritima*, which it is visited by insects only. To date only other species within the tribe, *Lavatera assurgentiflora*, is visited by hummingbirds in the Channel Islands (California, personal observation). In Europe where birds foraging on nectar are not frequent but not exceptional either, visits to *Lavatera arborea*, *Abutilon* sp. and *Malva sylvestris* by warblers have been reported (da Silva *et al.*, 2014), and to *L. maritima* by sparrows (personal observation). Besides its evolutionary distinctiveness, *Navaea* is a threatened taxon with low population effectives and possibly, low fecundity rates. It is therefore important to ascertain the extent of reproductive success mediated by current flower visitors. In this work the objectives were to provide an understanding of the ecology of pollination by birds and quantify its pollination effectiveness and to determine whether current visitors are able to provide a reproductive fitness that contributes to maintain the selection of ornithophilous floral traits. Therefore, we conducted a set of experiments and field censuses that accounted for both the quantitative component of pollination efficiency (i.e., the frequency of potential pollinators visits), and the qualitative component (pollen removal, deposition and fructification success) by insect and birds visitors. The aim of these works was to test the following hypotheses: 1) that present flower visitors drive enough reproductive fitness in male and female functions to maintain the selection of ornithophilous characters; 2) bird visitors are more efficient pollinators than insects, and 3) a certain pollen limitation is expected due to plant pollinator given if present bird visitors differ from the African bird species that exerted selection pressures in the past under the relict hypothesis.

Table 1. *Navaea phoenicea* subpopulations where reproductive experiments and visitor censuses were carried out. Asterisks denote populations where honeybee hives were detected.

Subpopulations in Anaga	UTM Coordinates	Subpopulations in Teno	UTM coordinates
Carboneras (CAR)	374938 3160920	Cecilia (CEC)	321141 3137625
Chinamada (CHN)	373241 3160579	Cuevas Negras (CVN)	320789 3138494
Taborno (TAB) *	375853 3160477	San Juan del Reparo (REP)*	316524 3137706
Tope Carnero (TOP)*	372112 3159782	Teno (TEN)*	316453 3137410

MATERIALS AND METHODS

Study site and species

Within the Tenerife Island, *Navaea phoenicea* has a restricted distribution with only two populations in the northern slopes of the oldest volcanic mountain massifs in the East (Anaga) and West (Teno) accounting for 15 subpopulations in total. Subpopulations selected for experiments and observations are listed in table 1. Primary habitat ranges from thermophilous shrubland up to the transition of the more humid laurel forests. The species is a big sized shrub with a flowering period spanning from the end of the aestival dry period in September until January, when blooming overlaps in time and space with *C. canariensis*. The big, reddish-orange flowers are displayed either isolated or in terminal racemes. All the experiments were conducted in three consecutive flowering periods between 2006 and 2009.

Flower experiments

To test the pollination success of flower visitors, a manipulation experiment of flowers was conducted. In each population the following treatment to random buds in each selected plant prior opening were applied: i) control (C), non-manipulated flowers; ii) autogamy (A), bagged flowers and no anther manipulation; iii) non-supplemented xenogamy (X), emasculated flowers; iv) supplemented xenogamy I (RX-I), emasculated flowers prior to anthesis and subsequently pollinated with stored pollen from other plant within the same subpopulation, upon the stylar branch exertion and bagged to control pollen source; v) supplemented xenogamy II (RX-II) was manipulated like RX-I but adding pollen from the opposite population. Treatments iv-vi included bagging with tulle boxes. Each treatment was applied if possible to two flowers per individual in five individuals, in eight subpopulations. Also, we attempted to set up a treatment with chicken wire to exclude birds but to allow insects in. However, in a preliminary experiment any wire diameter small enough to exclude birds seemed to deter insect visits, and therefore it was excluded. We did not include any other treatment as previous works already accounted for the breeding system features. Fruits were collected if produced. Fruit set was considered as the proportion of flowers setting fruits. Fruits were weighted and sowed under greenhouse conditions after manual scarification to account for viable seeds.

To evaluate differences in fructification success among treatments, the number of viable seeds per fruit and seed weight as an estimate of fitness were measured. Seed viability was assessed directly through germination after manual scarification. Failure or success of germination was considered as the response variable. Generalized linear mixed models (GLMM) with lme4 package (Bates *et al.*, 2013) implemented in R software (R Core Team, 2013) were applied to test for significant differences between treatments. Models included treatments, subpopulation and year as fixed factors and individual plants as random

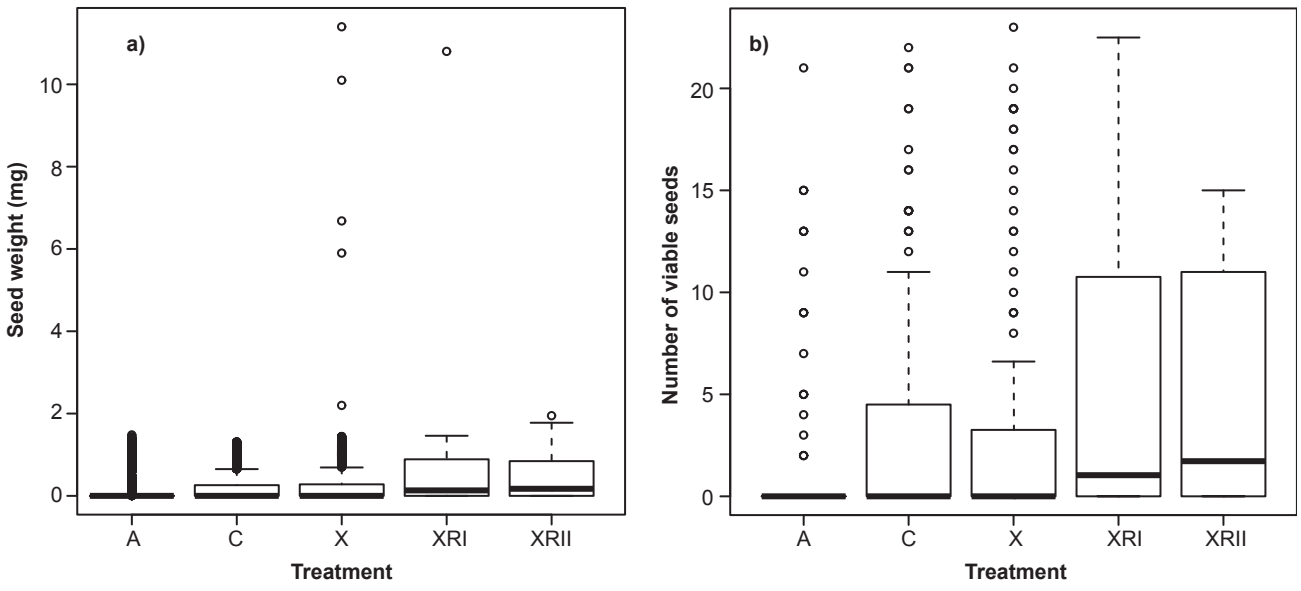


Figure 1. . Contribution of each treatment to a) Seed weight of fruit sets yielded by flowers, and b) Number of viable produced by each flowers. The complete flower set manipulated in each pollination treatment is accounted , indepentendly of whether the flower fructified or not. A= autogamy; C= control; X=xenogamy; XRI=reinforced xenogamy I; XRII= reinforced xenogamy II.

factor to control for potential individual variability in the response variables. We used a binomial distribution with a logit link function for germinating seeds and an exponential error distribution for seed weights. Differences between treatment means were tested pairwise through multiple comparisons using multcomp package (Hothorn *et al.*, 2008).

Frequency of visitors

Visitor censuses were conducted along the entire period of this work in all the subpopulations in different days across the flowering periods to account for variations between subpopulations or due to weather conditions. Observation times lasted for the whole sunlight period and occasionally during the night to discard visits by bats. The extent of the observation period ranged from 13 hours at the beginning of the flowering season to 10 at the end. Individual flowers were observed with binoculars from a distant discrete point to avoid interference with visitors. Five individuals could be controlled simultaneously. For every visit, we registered the time of the day, the species, the period of the visit, weather conditions, and type of visit: hovering, hanging or perching in the case of birds, and hovering or landing in the case of insects. Differences in the frequency of visitors and the effects of weather conditions in visit occurrence per species were tested with GLMM to test for significant differences between visitors with monitored individuals treated as random factors. Additionally, the daily pattern of visits was assessed with the overlap package (Meredith & Ridout, 2013) implemented in R. The total observation time spanned along 124 hours across 8 subpopulations in three years, 12 observation days in each subpopulation.

Pollen removal, transportation and deposition

Accounting for pollen loads carried by flower visitors was performed differently. Insects were captured after flower visits, stored in glass tubes and then immobilized with forceps during the manual count of pollen grains with a field magnifier. Insects were released after pollen count. Birds were captured with mist-nets placed between shrubs. The difficulty to place the nets due to steep habitats severely limited capture sessions and the number of nets to display. Captured birds were ringed with individual numbered rings plus colour rings employed to create unique combinations allowing subsequent visual differentiation of birds. Pollen loads were obtained from birds' heads with adhesive tape and pollen grains were counted afterwards in the laboratory. Differences in the number of grains were tested by means of linear models.

Table 2. Summary of statistical analysis for the results of manipulation experiments performed (a-b) and field observations of pollination efficiency measures (c-e).

a) Seed weight (n=5766)			
	df	**F**	**P**
Treatment	3	7.527	8.187*10-5
Year	2	0.444	0.506
Population	10	1.4405	0.198
b) Number of viable seeds per fruit (n=389)			
	df	**F**	**P**
Treatment	3	5.482	0.001
Year	2	0.130	0.719
Population	10	1.456	0.344
c) % of available pollen removed from anthers in one visit (n=217)			
	df	**F**	**P**
Species	7	33.513	2*10-6
Year	1	0.271	0.603
d) Number of pollen grains in an animal's body (n=116)			
	df	**F**	**P**
Species	7	1.865	0.0482
e) Number of pollen grains deposited on the styles in a single visit			
	df	**F**	**P**
Species	7	19.62	2*10-16
Style length	1	0.299	0.584

Flower buds were bagged prior to opening and were left open only during the monitoring period of the flower. For each flower, the number of visits and the number of anthers hosting pollen before and after each visits was controlled. To check for pollen deposition in the stylar branches, we emasculated and bagged flowers before style exertion and left opened when styles were exerted. Pollen grains were counted with a field magnifier and removed to allow pollen counting in the same style. Style length was measured to account for variation in deposition efficiency. Significant differences in pollen grains between visitors were tested with GLMs fitted with a Poisson distribution. The length of the monitored style was incorporated to the model, to account for possible effects of length on the number of grains deposited.

Fructification success

To assess the pollination effectiveness of bird species, emasculated flowers were bagged and left opened again when styles branches were exerted. After a single animal visit, flowers were bagged again, and fruits were collected if produced. Every year, the procedure was carried out until at least 40 flowers per visitor species were monitored. Seeds were weighted and subsequently sowed after manual scarification to determine germination success. GLMs were used to test the effect of the visitor species on the number of viable seeds per fruit and seed weight.

Pollination efficiency and landscape

The set of experiments performed allowed to determine both the Qualitative Components of pollination (QLC, proportion of grains removed, fruit set and number of viable seeds) and Quantitative Components (QNC, visitation frequency and number of flowers per plant) as defined in previous studies (Reynolds & Fenster, 2008; Rodríguez-Rodríguez *et al.*, 2013) providing therefore a direct comparison with the pollination efficiency of *Isoplexis canariensis* (Rodríguez-Rodríguez *et al.*, 2013), with a wider and more consistent temporal assessment of the five components, as all observation and experiments were carried out across three consecutive years. Every data frame of results for each component was resampled 5000 times following PE was calculated as the product of the mean values per sample, and this procedure was repeated

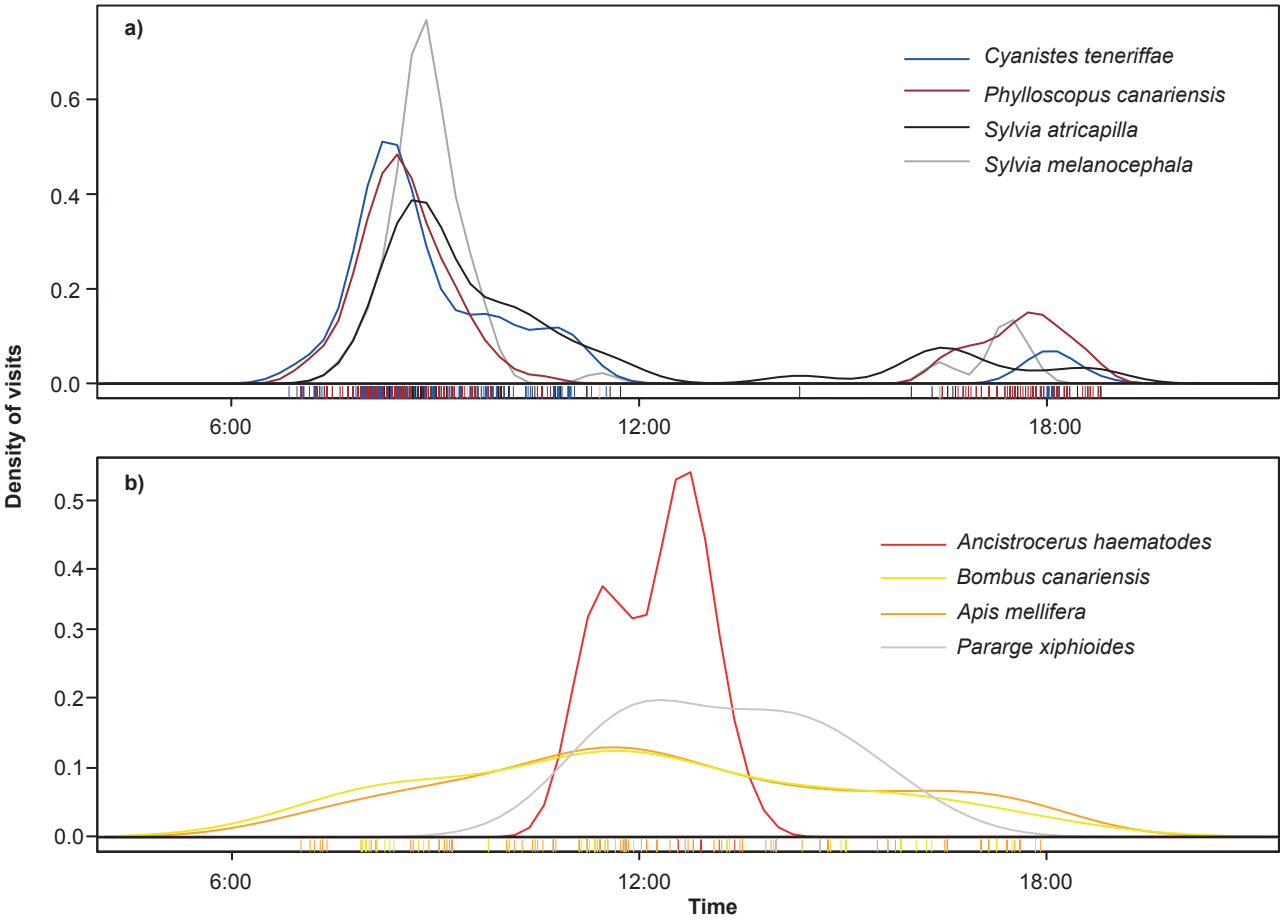


Figure 2. Daily visitation patterns of a) bird species and b) insect species in a 24 hours period. Visits are expressed as the density of visits in a given period of time.

5000 times to complete a data frame of PE mean values to account for mean and 95 CI limits. A complete description of the procedure is described in Rodríguez-Rodríguez *et al.* (2013).

RESULTS

Flower experiments

There were significant differences for both seed weight and number of viable seeds per fruit between treatments but not between years and populations (table 2a and b). Both supplemented cross pollination treatments yielded significant higher values for seed weight than non-supplemented outcrossing and control (figure 1a). Mean values of seed weight were 0.8 mg for A, 1.24 mg for C, 2.51 mg for X and 3.6 mg for XRI and 3.62 and for XRII. Likewise there were significant differences in the number of viable seeds per fruit (A=1.75, C= 2.94, X=4.75, XRI=6.51, XRII=6.6, figure 1b). Both results indicated pollen limitation. The similar values for non-supplemented outcrossing and controls along the lower seed yields of autogamy indicates that flower success occurred mostly by outcrossing.

Frequency of visitors

Bird frequency varied widely among species (df=7, F=3.971, P=0.001). Within birds, *Phylloscopus canariensis* and *Cyanistes teneriffae* showed a higher frequency of visits than warblers and insect species. The number of flowers visited in one single plane (geitonogamic visits) were significantly higher for *C. teneriffae* than the rest of bird species. Regarding insects, bumblebees and honeybees foraged repeatedly on the same plant before leaving (table 3a and b). One visit of *Serinus canarius* was reported in the observation period. *C. teneriffae* showed a higher rate of flowers visited per plant. Visitation rate of insects was still high as they accounted for 35% of the visits. Species*temperature and species*moisture interactions incorporated in a linear model were significant (df=7, F=1.34, P<0.001), indicating that species visitation rate differed depending on the environmental conditions. Because we did not detected insect activity below 11°C or above 85% humidity conditions, this indicated a weather limitation on the activity not detected in birds. The activity of *A. mellifera* was positively correlated with the presence of neighbour bee hives in TOP, REP, TAB and TEN (Monte-Carlo Mantel test, p<0.0012). No bats or lizards were detected.

Activity pattern of birds showed two clear activity peaks starting at dawn in the morning until 11:00 AM and a secondary short peak before dusk (figure 2a). Modes of visit differed significantly (p<0.0001) between *P. canariensis* and *C. teneriffae*. *P. canariensis* either hovered (65% of visits) or perched (35%). *C. teneriffae* hanged from the flower pedicel in most of the visits (78.74%) rather than perched (21.26%). Both warbler species perched in all observations. Insects used in all circumstances lower petals as landing platform and moved directly towards nectar receptacles. The visit pattern for insects in turn tended to concentrate the majority of visits at noon, possible because temperature conditions allowed a better insect movement.

Pollen removal, transportation and deposition

The mean proportion of anthers where pollen was retired in a single visit ranged from than 50% in *C. teneriffae* up to zero for *P. xiphioides* (table 3c, figure 3a). Bird species removed in each visit greater proportions of pollen than insects (table 2c). Posthoc tests revealed no differences in pollen removal between species within each of the two functional groups, birds and insects.

Table 3. Summary of the pollinator landscape components per species.

Visitor		a) flowers*plant^{-1}	b) Flower visits*30 min^{-1}	c) Percentage of anthers with pollen removed		d) number of grains in the body	
				Mean ± SD	n	Mean ± SD	Max
Birds	C. teneriffae	5.13±1.45	0.69±0.71	53.62±22.92	17	171.39±454.6	1476
	P. canariensis	1.27±0.12	0.75±0.84	51.12±21.56	10	21.6±35.95	106
	S. atricapilla	2.1±1.01	0.16±0.07	47.48±30.72	0	NA	NA
	S. melanocephala	2.0±1.01	0.25+0.17	55.07±28.16	1	15	15
Insects	A.haematodes	1.1±0.0	0.19±0.15	6.45±6.11	23	3.22±3.1	6
	Apis mellifera	8.3±1.7	0.32±0.43	3.76±3.75	34	2.26±2.87	8
	B. canariensis	9.1±0.6	0.30±0.18	2.06±3.31	19	2.37±3.02	8
	P. xiphioides	1.1±0.1	0.11±0.04	0	11	2.7±3.16	0

Bird ringing sessions shed poor sampling sizes of birds for the analysis of pollen transportation (figure 3b, table 3d). In total, we captured in mist-nets, 17 *C. teneriffae*, 11 *P. canariensis* and 1 *S. melanocephala* and no specimens of *S. atricapilla*. The amount of pollen grains found in the birds' heads varied widely. For *C. teneriffae* pollen loads ranged from 0 to 1476 grains and for *P. canariensis* from 0 to 106 grains. 58% of *C. teneriffae* specimens captured had pollen whereas 100% of *P. canariensis* had. Conversely the majority of insects captured showed very low pollen loads or no pollen at all. Pollen loads of *C. teneriffae* and *P. canariensis* were significantly higher than in insects, but with low power due to the scarce sampling (table 2d).

Birds deposited a significant higher amount of pollen grains than insects but the number of pollen grains showed high variations in every species and with a number of contact events with the stigma that left no pollen (tables 2e and 3e, figure 3c). Post hoc pairwise tests did not revealed differences among bird species and among insect species.

Fructification success

No flowers visited by insects fructified and were excluded from subsequent analyses. In birds, single bird visits yielded a fructification success of 3.87% in the flowers in *C. teneriffae*, 15.17% in *P. canariensis*, 18.42% in *S. atricapilla* and 18.75% in *S. melanocephala* (table 3f). GLMs performed for seed weights (df=3 F=10.17, p=1.27*10^{-6}) and number of viable seeds per fruit (df=3, F=4.562, p=0.006) indicated significant differences between bird species as well as for the interaction between number of pollen grains deposited by the flower visitor and the species. In particular, Tukey HSD post hoc tests indicated that visits of *C. teneriffae* yielded lower fructification rates than the rest of species.

Pollinator efficiency

Bootstrap estimation for averaging PE showed a coefficient of variation of 6.45% across iterations. Efficiency of birds differed widely between species and efficiency components and was low in all cases (figure 4). Quantity components where high in general and conversely quality components low. *P. canariensis* showed the highest PE score (0.028), with intermediate values for qualitative and quantitative components. *S. melanocephala* (PE=0.014) and *S. atricapilla* (PE=0.014) showed higher qualitative score and lower quantitative one, while on the opposite side, *C. caeruleus* showed the highest quantitative value and the lowest qualitative (PE=0.012) and was the least efficient pollinator. Scores for this species was due on the one hand to the high rate of geitonogamic visits and on the other to the low fructification rate driven. Insect PE score was in all cases zero due to the null qualitative contribution to reproductive fitness.

DISCUSSION

In this paper we assessed the entire spectra of the components intervening in the pollination process and the implications of each element for reproductive fitness. There were deep differences in both quantitative and qualitative aspects of the pollination landscape which can be interpreted on the basis of pollination behaviour. These results provide compelling evidence to exclude insects as legitimate pollinators but also allow detecting asymmetric values of the role of birds in the pollinator market. On the other side, they demonstrate that more than visitor census are needed to ascertain the pollination ecology of species, as the global pollination efficiency varies deeply depending on the results obtained for visit frequency. Assessments of pollinator shifts across plant lineages, pollination syndromes or networks which are based only in visit

Table 3. (Continuation)

Visitor		e) Pollen grains deposited		f) fructification success in one visit		
		Mean ± SD	Max	% of fruit sets	Seed weight (g)	Viable seeds/fruit
Birds	C. teneriffae	13.90±10.57	45	3.87	0.0013+0.0036	1.55+4.36
	P. canariensis	13.76±10.49	49	15.170	0.0028+0.0051	3.62+6.33
	S. atricapilla	15.21±17.52	67	18.420	0.0033+0.0058	4.59+7.17
	S. melanocephala	10.69±12.21	40	18.75	0.003+0.0054	4.07+6.51
Insects	A.haematodes	1.27±1.60	5	NA	NA	NA
	Apis mellifera	0.62±0.91	3	NA	NA	NA
	B. canariensis	2.40±2.61	8	NA	NA	NA
	P. xiphioides	1.5±1.75	5	NA	NA	NA

observation or incomplete knowledge of plant pollinator interactions may risk to be biased.

Pollination efficiency of flowering visitors

The analysis of pollination effectiveness components shed very different results for both birds and insects. Insect species revealed as mere nectar robbers with a null contribution to reproductive fitness. Their visit frequency was however very significant for native *Bombus* and introduced *Apis*, which only occurred in subpopulations close to hives. Even if a consistent daily pattern of visits was found, limiting the activity to the warmest hours in the middle of the day, this was dependent on weather conditions. The most normal meteorology consists in humid and cool days which will not generally favour insect visits. Nevertheless, the frequency of visits is the highest recorded for a MBE species, and what it is therefore unknown is the reason of why insects are attracted by a red flower putatively adapted to bird pollination.

Insects presented a null contribution to reproductive success. All the species were found to remove, transport and deposit very low amounts of pollen which translated into fructification failure. *Navaea phoenicea* flowers are big sized and open. In most visits, insects were able to land directly in lower petals and moved towards nectar receptacles without contacting staminal column or stylar branches. Hence, pollination accuracy was null for insect species. As pollen is shed rapidly after anthesis, a part of it falls into the nectar receptacles. Therefore it is likely that most pollen grains carried by insects are attached after contact at the basis of petal not the staminal column.

The results of flower manipulation experiments and quantification of the six parameters of pollination efficiency showed great differences across the elements analysed. In one extreme, both warblers showed the highest quality pollination, whereas in the other blue tits had the highest amount of visits. Manipulation experiments yielded higher fruit sets in pollen supplemented treatments than outcrossing alone, indicating a pollen limitation. Considering visitation patterns and frequency, the four detected species had two clear peaks of activity at dawn and dusk as a general term. This is at odds to the pattern found in *Isoplexis canariensis*, where *P. canariensis* had a higher frequency in the evening. Both warbler species showed much lower frequency with respect to *P. canariensis* and *C. caeruleus*, which is likely to be due the lower abundance estimated for these two species in Tenerife (Martín & Lorenzo, 2001). Compared to other ornithophilous species, *P. canariensis* and *C. teneriffae* had higher visitation frequencies than when foraging on *Canarina canariensis* (Rodríguez-Rodríguez & Valido, 2011), *Isoplexis canariensis* (Rodríguez-Rodríguez & Valido, 2008), *Lotus* (Ollerton *et al.*, 2009) or *Scrophularia* spp. (Ortega-Olivencia *et al.*, 2012). Conversely visit rates in the continental *Anagyris latifolia* were higher, especially in warbler species (Valtueña *et al.*, 2008). Beyond the abundance of visitors, other factors that may explain the differences between species can be also considered. For instance, food availability, like insects or fruits, could be scarcer for passerines during the flowering period of *Navaea phoenicea* and therefore species may rely more on nectar supply from flowers. Indeed, across our field work we observed little coincidence with other plant food sources such as fruits or, certainly, other flowers potentially attractive to birds. Therefore, these floral traits may be better perceived in the isolation of the winter plant landscape. For some reason, the flowers of *Navaea phoenicea* do not attract lizards species, contrary to what occurs in other species. With similar frequency of visits in *C. caeruleus* and *P. canariensis*, the calculated quantitative component of the PE was much higher in the first due to geitonogamic visits. Although this is considered in the final product of the PE, geitonogamy is not necessarily qualitatively adaptive for the fitness of the plant as it does not promote outcrossing. Indeed previous results show that outcrossing has facilitated a higher genetic diversity despite low effective numbers and geographical disjunction limiting gene flow. Further criticism to the PE calculation approach is provided below.

The quality components of the pollination landscape were different between bird species as well. The difficulty of bird captures in the habitat yielded poor sampling size, which therefore limiting the information about pollen loads. The majority of birds carried pollen in the forehead but the amounts counted varied greatly. Pollen load was greater in blue tits in which thousands of pollen grains were counted twice within a small sample size of captured birds. We did not find those numbers for chiffchaffs, but this is likely due to sampling limitations, as during visit censuses big pollen loads in the head of chiffchaffs could be observed. This issue was observed in warblers, but with a lower frequency. The four species showed similar values of pollen removal and deposition, indicating that their efficiency for both parameters was similar. All species showed to be able to achieve a complete removal in one visit

the whole pollen hosted in the staminal column, although the amounts of pollen removed varied widely. A similar finding was obtained for the number of grains deposited in stylar branches, which was not significantly different between bird species either. However, the mean number of grains deposited was more frequently below the mean number of ovules in the flowers (21, chapter 3), indicating that most part of the visits may not assure fructification success. This is consistent with the fructification success results. As for the deposited grains, *S. atricapilla* drove the highest fructification rate. Blue tits in turn drove a very low fructification success. With similar quality components to other species, plus very high pollen loads there was not a priory any expectation on significantly lower rates of fructification success. This failure can be interpreted on the basis of the foraging behaviour of blue tits. In most of the visits, blue tits hanged from the pedicel to manipulate the petals and the staminal column with the legs to reach all the nectar receptacles (figure 5a), which is an extended foraging behaviour in blue tits (Carrascal *et al.*, 1994). Unlike what it is described for ornithophilous flowers (Cronk & Ojeda, 2008), the pedicels of the flowers appear to be weak and unlikely to stand heavy weights or such manipulations by birds. In consequence, the visits of *C. teneriffae* appear to damage flowers, precluding the completion of the pollination and fructification process. These results are based on the success of a single visit. While for other species a cumulative number of visits on a flower can increase the probability of fructification, a blue tit visit may damage a pollinated flower. On the other hand,

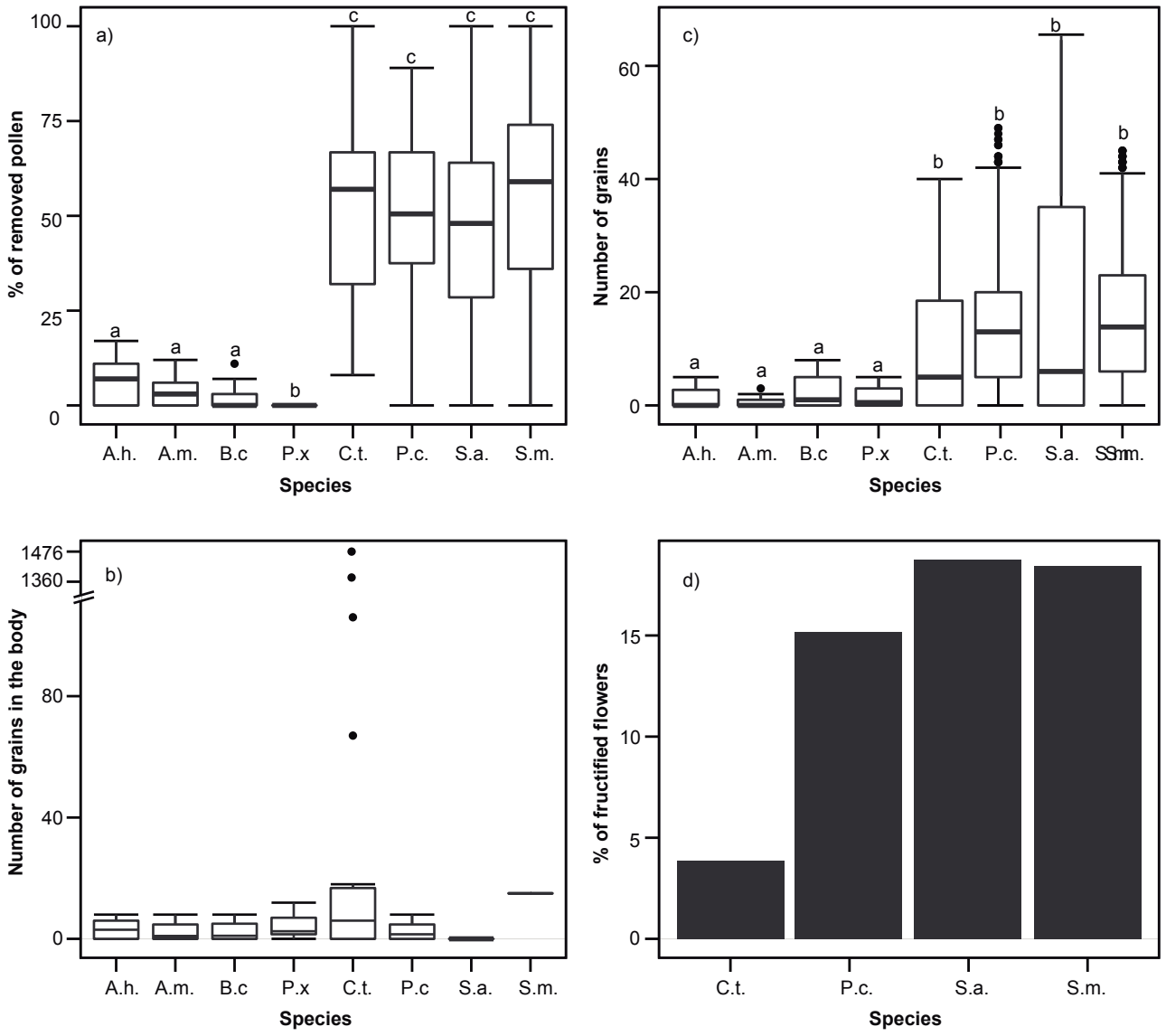


Figure 3. Contribution of each flower visitor to a) male fitness, expressed as the percentage of pollen removed from anthers per visit; b) Number of pollen grains on the body of captured animals; c) Number of grains deposited on stigmas per visit; d) Percentage of visits that result in successful fructification. Letters above boxplots denote statistical groups. Insects: A.h.=*Ancistrocerus haematodes*,; A.m.= *Apis mellifera*; B.c.= *Bombus canariensis*; P.x.= *Pararge xiphioides*. Birds: C.t.=*Cyanistes teneriffae*; P.c.=*Phylloscopus canariensis*; S.a=*Sylvia atricapilla*; S.m.= *Sylvia melanocephala*.

it is interesting that the position of flowers in branches appears to favour perching rather than hovering as flowers are most commonly arranged in racemes, orientated upwards and show a slight, but patent zigomorphy as the staminal column is slightly curved upwards (Cronk & Ojeda, 2008), along with floral orientation which can affect to visitors behaviour as well (Fenster *et al.*, 2009). While hovering has been traditionally attributed to specialist nectarivorous birds, mostly exclusively to hummingbirds, it is not an infrequent behaviour among passerines in the Old World (Wester, 2014), which has been observed across Iberian and Macaronesian ornithophilous species in both chiffchaffs and warblers. Even sunbirds show effective hovering behaviour (Janeček *et al.*, 2011). Here, hovering is the most frequent pattern in *P. canariensis*, but not in *Sylvia* species (figure 5b) which are unable to sustain for the needed time to drink nectar. When capturing birds, it was also notable that pollen loads in chiffchaffs were limited to the forehead while in blue tits the pollen was also found in beak and throat, probably as a result of the behaviour pattern. Foraging behaviour differences may then drive the different fructification rates found between warblers and chiffchaffs. The latter showed a higher PE score, but it remains unknown whether if ecological conditions allowed a higher density of warblers, this would increase the reproductive fitness in *N. phoenicea*.

The analyses of qualitative and quantitative components allowed for a direct comparison between flower visitors, but interpretation of differences between plant species should be taken with caution. Any of the visitors of *Navaea phoenicea* showed a much lower score than any of those visiting *Isoplexis* flowers (Rodríguez-Rodríguez *et al.*, 2013), and the distribution of the two components is deeply different. *Isoplexis* showed moderate values for both components, whereas *Navaea* showed much lower quality scores and much higher quantitative ones. The most important difference was the restriction

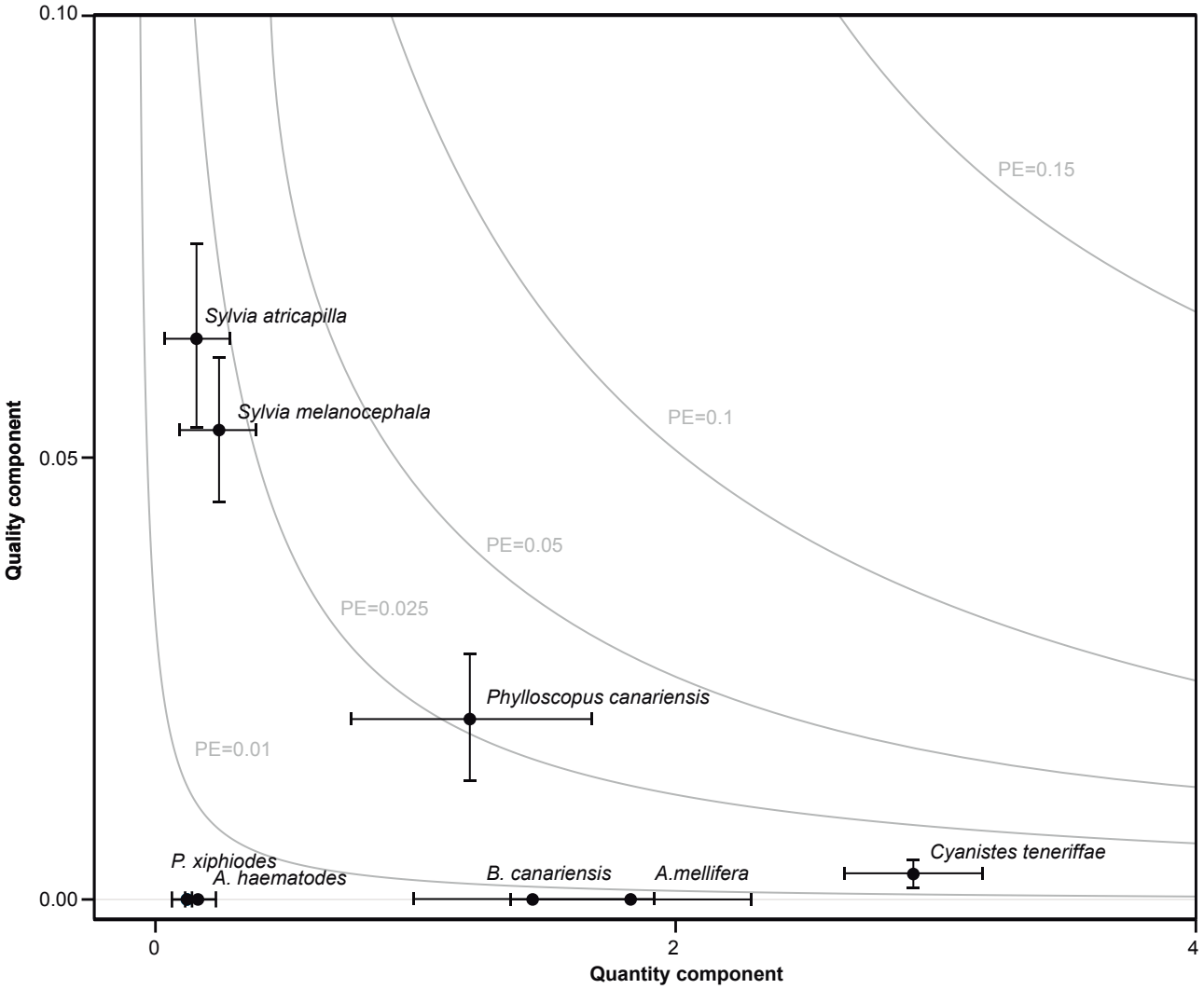


Figure 4. Pollination Efficiency values for the eight species, expressed as the mean ± standard deviation for quantitative component (x axis) and qualitative component (y axis). Grey lines denote isolines for PE values.

of pollinators in *N. phoenicea* to only one functional group, birds, while *Isoplexis* counted with both lizards and birds groups. Within the birds, three out of fours species demonstrated to be true pollinators, but with different roles across the pollinator landscape. This redundancy in species mitigate the breakdown of plant-pollinator networks. This approach, however, may not give insights enough to argue that reproductive fitness is higher in one species than in other. A number of reasons for most of the quantified components is given: 1) frequency of visits is influenced by several ecological constraints that are not related to attraction cues and adaptation of floral traits such as local variations of pollinator abundances (e.g. Gómez & Zamora, 1999; Herrera, 2005). 2) As argued above, geitonogamic visits do not necessarily provide a higher fitness for the population; 3) proportions of viable seeds depend on other factors not related to flower visitors. Self-compatibility can indeed influence seed viability, hence geitonogamy may again not be adaptive in self-incompatible species; 4) even if the proportion of pollen removed is accounted as an accurate measure of the fitness of the male function, there is not a correspondent measure to estimate the female function, because the number of pollen grains deposited in the stigmas by the same visitor is not comparable among different taxa. Finally, the pollination efficiency result consists merely in the product of five decimal numbers, where a slight difference in one of the components can drive differences of several orders of magnitude between species, hampering the interpretation of the final PE outcome. We therefore suggest that an

Figure 5. Pictures of bird visitors: a) Blue tit hanging on the flower in the search of nectar; b) captured Blue tit with carrying pollen grains on the head (n=1476) c) blackcap warbler foraging in *Navaea phoenicea* in a perching position; d) Canarian chiffchaff perching to drink nectar.

approach that accounts for standardised fitness measures of male and female components of pollination, multiplied by the frequency of visits may provide a forward step to carry out quantitative comparisons.

Insights for the bird-flower mutualism

These results provide the first understanding of the contribution of generalist passerines to the reproductive assurance of *Navaea phoenicea*. On the one hand, the counterproductive role of *C. teneriffae* leads to discard a recent pollination mutualism. Foraging episodes by *C. teneriffae* can be simply considered exclusively as an opportunistic behavior, like it has been detected in other passerines across islands and continents. Even though it may not be a generalized pattern, flower interactions with *C. teneriffae* occur independently of a potential density compensation process in the Macaronesian islands. On the other hand, two hypotheses can be enunciated for *P. canariensis* and *Sylvia spp.* on the basis of the different qualitative and quantitative contributions to the reproductive success of the species. First, *P. canariensis* are proved here to be legitimate and predictable visitors and, to a certain point, an efficient pollinator. As a general rule, papers focused on the Mediterranean and Macaronesian ornithophilous taxa consistently identify *P. canariensis s* as a true pollinator (Rodríguez-Rodríguez & Valido, 2008, 2011; Ortega-Olivencia *et al.*, 2012). The question of to what extent the pollination effectiveness of *P. canariensis* alone is enough to maintain in such endangered plant species highlights the need of a demographic assessment to place in the conservation context the contribution of pollinators to the persistence of populations. The phylogeny for the Malveae tribe designates *N. phoenicea* as an ancient insular species (Escobar García *et al.*, 2009) for which the relict hypotheses remains as the most plausible. Our findings in this paper deepen into the idea that generalist passerines maintain at present the selective pressure on traits related to bird pollination, as we could identify the contribution for each of them. The origins of the Macaronesian by avifauna are generally recent (Illera *et al.*, 2012). The colonization of the Canarian archipelago by *Phylloscopus* occurred the earliest 2.5 Mya (Olesen *et al.*, 2012). The pollination syndrome in *Navaea phoenicea* is likely to be originated in the African continent earlier, so the possible existing gap between original pollinators and the transitional ones remains unexplained. However the evolution of bird flower mutualisms in *Canarina canariensis* lineage has been associated to the colonization of the islands (Olesen *et al.*, 2012). The second hypothesis accounts for the high rates of fructification achieved by warblers due to an accurate pollination process by perching. Because the frequency of visits can be attributed to other factors other than attraction, such as density, their role cannot be dismissed. Under a scenario of higher density of individuals, these frugivorous warblers might drive higher fructification rates thus exerting a strong selection pressure. Therefore the *de novo* hypothesis is not necessarily discarded from an ecological perspective. It is notable here that with lower observation numbers, warblers would have remained undetected as floral visitors and hence their role discarded. From a phylogeographic perspective however, Canarian warblers only colonized the archipelago recently (Dietzen *et al.*, 2008), leaving the chronological breach undisclosed as well. The high redundancy that is provided by the different species, with different roles across the pollinator landscape suggest that such level of generalization may be considered as optimal (Gómez *et al.*, 2007). Therefore, the selective pressure on floral traits may be exerted jointly by the assemble of true pollinators rather than by the individual reproductive contribution of each bird species.

The knowledge gap existing about the early steps in the colonization of the islands and the extinction of putative vertebrate species hinders at the present time any deeper interpretation of the evolution of pollinator syndromes in the island. However a number of useful insights can be obtained from a deeper examination of floral traits to enunciate specific hypotheses about their evolution in response to selective pressure by pollinators. For instance, flower color has been only studied for *Lotus berthelotii*, *Isoplexis canariensis and Canarina canariensis*, but proving better visibility of flowers for birds than for insects, yet the color sensitivity of birds is only known at present for the continental *C. caeruleus* (Hart, 2001). On the other hand, several questions about nectar as the principal reward for visitors have still to be solved. It has been postulated that nectar sugar composition in the MBE has evolved in response to the ability of generalist passerines to digest sugar (Dupont *et al.*, 2004). This has been considered at a first sight, at odds with the relict hypotheses, although nectar sugar composition is a labile trait. Nevertheless, these hypotheses lack some basic knowledge about passerines digestion ability, actual nectar production and sugar composition variation or the presence of important components of nectar such as amino acids. The exploration of these properties in response to flower visitors can help to

advance further in the evolution of bird flower interactions in the Canary Islands.

Concluding remarks

In this paper we demonstrated the differentiated efficiency of flower visitors according to different pollination components. These findings have several implications, the first of all the importance of the exploration of the different components of the pollination process. This work demonstrates a strong need to account for the whole ensemble of components of the pollination process to ascertain the evolution of pollination interactions. Moreover, these results constitute a first element of analysis of the evolution of bird flower interactions in *Navaea phoenicea*, which leads to address other components of the ornithophilous syndrome as well as the implications for the conservation of an endangered, distinct species.

REFERENCES

Anderson S.H. (2003) The relative importance of birds and insects as pollinators of the New Zealand flora. *New Zealand Journal of Ecology*, **27**, 83–94.

Bates D., Maechler M., & Bolker B. (2013) lme4: Linear mixed-effects models using S4 classes. Version 0.9.

Carrascal L.M., Moreno E., & Valido A. (1994) Morphological evolution and changes in foraging behaviour of island and mainland populations of Blue Tit (*Parus caeruleus*)—a test of convergence and ecomorphological hypotheses. *Evolutionary Ecology*, 25–35.

Castro-Urgal R. & Traveset A. (2014) Differences in flower visitation networks between an oceanic and a continental island. *Botanical Journal of the Linnean Society*, **174**, 478–488.

Cronk Q. & Ojeda D.I. (2008) Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, **59**, 715–727.

Dietzen C., Garcia-Del-Rey E., Castro G.D., & WINK M. (2008) Phylogenetic differentiation of *Sylvia* species (Aves: Passeriformes) of the Atlantic islands (Macaronesia) based on mitochondrial DNA sequence data and morphometrics. *Biological Journal of the Linnean Society*, **95**, 157–174.

Dupont Y.L., Hansen D.M., Rasmussen J.T., & Olesen J.M. (2004) Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. *Functional Ecology*, **18**, 670–676.

Escobar García P., Schönswetter P., Fuertes Aguilar J., Nieto Feliner G., & Schneeweiss G.M. (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). *Molecular Phylogenetics and Evolution*, **50**, 226–39.

Faegri K. & Van Der Pijl L. (1979) *The principles of pollination ecology*. Elsevier, Oxford.

Fenster C.B., Armbruster W.S., & Dudash M. (2009) Specialization of flowers: is floral orientation an overlooked first step? *New Phytologist*, **183**, 502–506.

Gómez J.M., Bosch J., Perfectti F., Fernández J., & Abdelaziz M. (2007) Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia*, **153**, 597–605.

Gómez J.M. & Zamora R. (1999) Generalization vs. specialization in the pollination system of *Hormatophylla spinosa* (Cruciferae). *Ecology*, **80**, 796–805.

Hart N.S. (2001) The visual ecology of avian photoreceptors. *Progress in Retinal and Eye Research*, **20**, 675–703.

Herrera C.M. (2005) Plant generalization on pollinators: species property or local phenomenon? *American Journal of Botany*, **92**, 13–20.

Hothorn T., Bretz F., & Westfall P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Illera J.C., Rando J.C., Richardson D.S., & Emerson B.C. (2012) Age, origins and extinctions of the avifauna of Macaronesia: a synthesis of phylogenetic and fossil information. *Quaternary Science Reviews*, **50**, 14–22.

Janecek Š., Patácová E., Bartoš M., Padyšáková E., Spitzer L., & Tropek R. (2011) Hovering sunbirds in the Old World: Occasional behaviour or evolutionary trend? *Oikos*, **120**, 178–183.

Martín A. & Lorenzo J.A. (2001) *Aves del archipiélago canario*. Francisco Lemus Editor, La Laguna, Tenerife.

Mayr G. (2004) Old World fossil record of modern-type hummingbirds. *Science (New York, N.Y.)*, **304**, 861–864.

Mayr G. & Wilde V. (2014) Eocene fossil is earliest evidence of flower-visiting by birds Eocene fossil is earliest evidence of flower-visiting by birds. *Biology Letters*, **10**, 1–4.

Meredith M. & Ridout M. (2013) Overlap: Estimates of coefficient of overlapping for animal activity patterns. Version 0.24.

Navarro-Pérez M.L., López J., Fernández-Mazuecos M., Rodríguez-riaño T., Vargas P., & Ortega-Olivencia A.

(2013) The role of birds and insects in pollination shifts of *Scrophularia* (Scrophulariaceae). *Molecular Phylogenetics and Evolution*, **69**, 239–254.

Nogales M., Heleno R.H., Traveset A., & Vargas P. (2012) Evidence for overlooked mechanisms of long-distance seed dispersal to and between oceanic islands. *New Phytologist*, **194**, 313–317.

Ojeda D.I. (2013) The Macaronesian bird-flowered element as a model system to study the evolution of ornithophilous floral traits. *Vieraea*, **41**, 73–89.

Ojeda D.I., Santos-Guerra A., Oliva-Tejera F., Valido A., Xue X.., Marrero-Rodríguez Á., Caujapé-Castells J., & Cronk Q. (2013) Bird-pollinated Macaronesian *Lotus* (Leguminosae) evolved within a group of entomophilous ancestors with post-anthesis flower color change. *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 193–204.

Olesen J.M. (1985) The Macaronesian bird-flower element and its relation to bird and bee oppurtunists. *Journal of the Linnean Society*, **91**, 395–414.

Olesen J.M., Alarcón M.L., Ehlers B.K.B.K.., Aldasoro J.J., & Roquet C. (2012) Pollination, biogeography and phylogeny of oceanic island bellflowers (Campanulaceae). *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 169–182.

Olesen J.M., Eskildsen L.I., & Venkatasamy S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, **8**, 181–192.

Olesen J.M. & Valido A. (2003) Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution*, **18**, 177–181.

Ollerton J., Cranmer L., Stelzer R.J., Sullivan S., & Chittka L. (2009) Bird pollination of Canary Island endemic plants. *Die Naturwissenschaften*, **96**, 221–32.

Ortega-Olivencia A. & Catalán P. (2009) Systematics and evolutionary history of the circum-Mediterranean genus *Anagyris* L. (Fabaceae) based on morphological and molecular data. *Taxon*, **58**, 1290–1306.

Ortega-Olivencia A., Rodríguez-Riaño T., Pérez-Bote J.L., López J., Mayo C., Valtueña F.J., & Navarro-Pérez M.L. (2012) Insects, birds and lizards as pollinators of the largest-flowered *Scrophularia* of Europe and Macaronesia. *Annals of botany*, **109**, 153–67.

Ortega-Olivencia A., Rodríguez-Riaño T., Valtueña F.J., López J., & Devesa J.A. (2005) First confirmation of a native bird-pollinated plant in Europe. *Oikos*, **110**, 578–590.

R Core Team (2013) R: A Language and Environment for Statistical Computing. Version 3.1.2.

Reynolds R.J. & Fenster C.B. (2008) Point and interval estimation of pollinator importance: a study using pollination data of *Silene caroliniana*. *Oecologia*, **156**, 325–32.

Rodríguez-Rodríguez M.C., Jordano P., & Valido A. (2013) Quantity and quality components of effectiveness in insular pollinator assemblages. *Oecologia*, **173**, 179–90.

Rodríguez-Rodríguez M.C., Jordano P., & Valido A. (2015) Hotspots of damage by antagonists shape the spatial structure of plant-pollinator interactions. *Ecology*, **96**, 2181–2191.

Rodríguez-Rodríguez M.C. & Valido A. (2008) Opportunistic nectar-feeding birds are effective pollinators of bird-flowers from Canary Islands: Experimental evidence from *Isoplexis canariensis* (Scrophulariaceae). *American Journal of Botany*, **95**, 1408–1415.

Rodríguez-Rodríguez M.C. & Valido A. (2011) Consequences of plant-pollinator and floral-herbivore interactions on the reproductive success of the Canary Islands endemic *Canarina canariensis* (Campanulaceae). *American Journal of Botany*, **98**, 1465–1474.

da Silva L.P., Ramos J.A., Olesen J.M., Traveset A., & Heleno R.H. (2014) Flower visitation by birds in Europe. *Oikos*, **123**, 1377–1383.

Traveset A., Tur C., Trøjelsgaard K., Heleno R.H., Castro-Urgal R., & Olesen J.M. (2015) Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*, **24**, 1–11.

Trøjelsgaard K., Báez M., Espadaler X., Nogales M., Oromí P., Roche F. La, & Olesen J.M. (2013) Island biogeography of mutualistic interaction networks. *Journal of Biogeography*, **40**, 2020–2031.

Trøjelsgaard K. & Olesen J.M. (2013) Macroecology of pollination networks. *Global Ecology and Biogeography*, **22**, 149–162.

Valido A., Dupont Y.L., & Olesen J.M. (2004) Bird-flower interactions in the Macaronesian islands. *Journal of Biogeography*, **31**, 1945–1953.

Valido A. & Nogales M. (2003) Digestive ecology of two omnivorous Canarian lizard species (*Gallotia*, Lacertidae). *Amphibia Reptilia*, **24**, 331–344.

Valtueña F.J., Ortega-Olivencia A.N.A., Rodríguez-Riaño T., & López J. (2008) Reproductive biology in *Anagyris foetida* L. (Leguminosae), an autumn-winter flowering and ornithophilous Mediterranean shrub. *Botanical Journal of the Linnean Society*, **157**, 519–532.

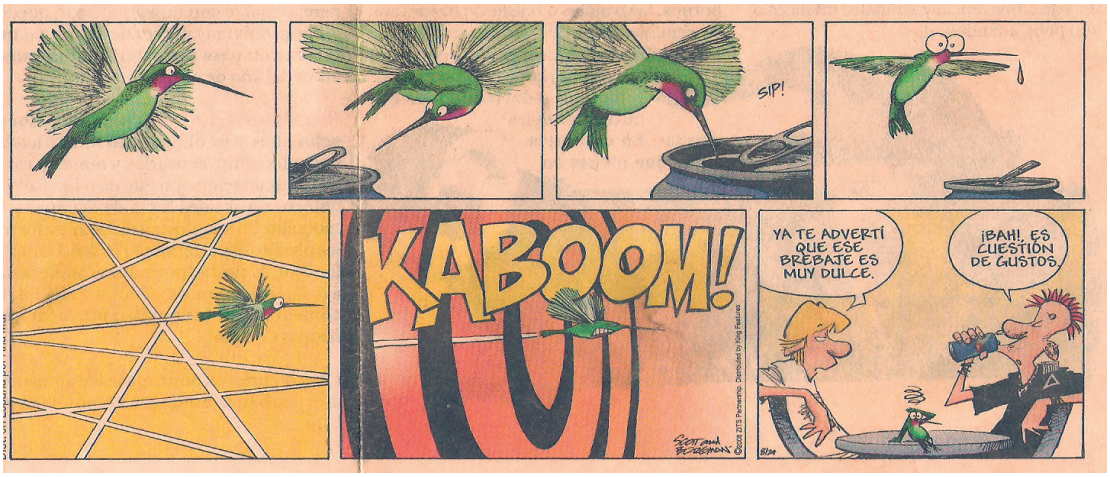
Vogel S., Westerkamp C., Thiel B., & Gessner K. (1984) Ornitophilie auf den Canarischen Inseln. *Plant Systematics and Evolution*, **146**, 225–248.

Waser N.M., Chittka L., Price M. V, Williams N.M., & Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.

Wester P. (2014) Feeding on the wing: Hovering in nectar-drinking Old World birds - More common than expected. *Emu*, **114**, 171–183.

5 Attraction cues and nectar composition reveal why insects keep visiting Macaronesian ornithophilous endemic *Navaea phoenicea* (Malvaceae)

Las señales de reclamo y la composición del néctar revelan por qué los insectos visitan el endemismo ornitófilo Macaronésico *Navaea phoenicea* (Malvaceae)



5. Attraction cues and nectar composition reveal why insects keep visiting Macaronesian ornithophilous endemic *Navaea phoenicea* (Malvaceae)

Attraction cues and nectar composition reveal why insects keep visiting Macaronesian ornithophilous endemic *Navaea phoenicea* (Malvaceae)

ABSTRACT

Shifts in the assignment of ensembles of floral traits to different pollinators determine their role in mutualistic networks. Within the Macaronesian flora, members of the "Macaronesian ornithophilous element" have appeared independently within different families showing flower traits adapted to bird pollination. Our study focuses on pollination visits and color traits for attraction and nectar reward in natural populations of the relict Canarian endemic *Navaea phoenicea* (Malvaceae). Patterns of nectar production, sugar concentration and composition were assessed. Color was analyzed to explore the cryptic chromaticity with respect to birds and insects visual systems with reflectance measurements of individual organs, background vegetation and flowers of co-flowering species. Finally, the type of epidermal cells in the petals was assessed to determine the existence of mechanic barriers to insects. Flowers showed high daily amounts of diluted nectar with only the volume varying across flowering stages, but not the concentration. Nectaries from upper petals yielded higher volumes. Volume varied significantly across years and removal significantly increased the total amount. Composition was glucose and fructose, with significant variability between nectary position and individuals. Such variation did not affect the suite of pollinators. Reflectance spectra were similar across parts of the petals and other floral organs. However profiles showed two peaks: one in the UV-blue wavelength and another stronger associated to red reflectance. The color and nectar volumes are consistent with the bird pollination syndrome, but the UV-blue reflectance peak matches those found in other enthomophilous mallows. Within the anthocyanin chemical suite, red pigments may be an adaptation to bird pollination and UV-blue second peak explains the flower attraction for insects. The presence of tabular-rugose cells did not act as a barrier to insects. In conclusion, floral traits are exclusively adapted to attract birds, but not to deter illegitimate insects.

5. Attraction cues and nectar composition reveal why insects keep visiting Macaronesian ornithophilous endemic *Navaea phoenicea* (Malvaceae)

INTRODUCTION

Plant-animal interactions are one of the major forces driving evolution and diversification in flowering plants. Among mutualistic interactions, selection in pollinator-niche acts across different taxa directly on sets of floral traits (Van der Niet & Johnson, 2012). Changes in pollinator niches among specialized plants are frequent, because they are defined by a principal pollinator type that will cause adaptive pollinator-mediated divergence in floral traits (Van der Niet *et al.*, 2014). This has led to adopt the longstanding concept of pollination syndromes, defined as the shared set of floral traits in a given group of species evolved in response to selective pressures of a particular type of pollinator (Fenster *et al.*, 2004). Even though the concept has been frequently challenged, it clearly serves to summarize as set of common traits important to ensure an efficient plant-pollinator interaction (Faegri & Van Der Pijl, 1979; Waser *et al.*, 1996; Ollerton, 2014; Rosas-Guerrero *et al.*, 2014). Among different pollination syndromes (Faegri & Van Der Pijl, 1979), ornithophily is the ensemble of traits including large-sized, non-scented, long-lived flowers displaying reddish-colored corollas, which provide large amounts of viscous nectar, with flower shape and orientation varying depending to the group of birds (Cronk & Ojeda, 2008).

Whereas bird-flower interactions are more common in tropical continental habitats than in other biogeographic regions, it is remarkably present in oceanic islands, where it has been interpreted as a response to food scarcity and the result of trophic niche expansion (Trøjelsgaard *et al.*, 2013). One of such cases is found in the Macaronesian region, an ensemble of five volcanic archipelagos in the Atlantic Ocean. Here, at least 16 species in eight different plant families conform the so-called "Macaronesian bird flower element" (MBE), which in different degrees share a suite of floral traits associated to bird pollination by local generalist passerines (Ojeda, 2013). In the Canary Islands, the most frequent passerine species foraging on flowers are Canarian blue tits (*Cyanistes teneriffae*, Paridae), chiffchaffs (*Phylloscopus canariensis*, Phylloscopidae) and warblers (*Sylvia atricapilla*, *S. melanocephala* and *S. conspicilliata*, Sylviidae) (Olesen & Valido, 2004; Rodríguez-Rodríguez & Valido, 2011; Rodríguez-Rodríguez *et al.*, 2013). A considerable effort has been made so far to disentangle the origin of the MBE (Ojeda, 2013). Most of the current knowledge on the subject is centered on the analyses of pollinator effectiveness, on the phylogenetic patterns for evolution of pollination syndromes and on morphological floral traits in different MBE lineages (Ojeda, 2013; Ojeda *et al.*, 2013). However, the knowledge on how these convergent traits ensure plant pollinator mutualisms is incomplete. The fundamental steps of attraction cues, pollinator reward and exploitation barriers require deep and integrated studies at the species level. To date, in the MBE there are no detailed studies on a single species comprising the ensemble of floral traits pertinent for pollination interactions. These traits are: 1) color and light reflectance; 2) amount of nectar secretion, concentration and composition, and 3) the mechanic strategies that facilitate or deter flower visitors.

First, color relates directly to the perception of visual stimulation by visitors. Red flowers are commonly associated to bird pollination visual system (Shrestha *et al.*, 2013), but there is also growing evidence that red color can serve as a mechanism to hide flowers from illegitimate insects (Bergamo *et al.*, 2015). Nevertheless, flower spectra may be complex and context-dependent (see Chittka Raine, 2006 for review). At present only analytical examination of color spectra and the perception by birds and insects has been conducted in three MBE species: *Canarina canariensis*, *Isoplexis canariensis* but only exhaustively in *Lotus berthelotii* and relatives (Ollerton *et al.*, 2009; Ojeda *et al.*, 2013).

The second trait, nectar, differs between species in amount and chemistry in response to selective pressures exerted by pollinators. Pollination by Neotropical hummingbirds and African sunbirds favors sucrose-type nectar, whilst it is assumed generalist passerines that forage occasionally on flowers tend to select for diluted hexose-type nectar (Nicolson, 2002; Johnson & Nicolson, 2008). However, this hypothesis cannot be generalized, as we ignore the ability for sucrose digestion of most nectar-feeding birds. In particular, in most of the passerine families foraging on flowers sucrose digestion ability is actually present (Lotz & Schondube, 2006) and specialized birds like sunbirds do not show any preference for hexose or sucrose nectars (Nicolson & Fleming, 2003). Although there is some empirical support for this dichotomy between bird pollinated flowers containing hexose-type nectar and insect pollinated flowers containing sucrose-type (Dupont *et al.*, 2004a), nectar dynamics of the species and variability of concentration, sugar composition or volume have not been studied in detail for most of the MBE species (but see Valtueña *et al.*, 2007).

Finally, the mechanic interaction between flower and visitors during flower visitation has only started to be explored recently by assessing cellular adaptations in petal epidermal surfaces in a phylogenetic context (Cronk & Ojeda, 2008). A common adaptation to preclude illegitimate visitors to forage on nectar is the corolla obturation. This might not be possible in many ornithophilous species which may require open corollas, but other physical adaptations may appear. Specifically, in *Lotus* a shift in petal epidermal cell types, from papillose to tabular cells has been associated to the change in pollination system from insect to bird, which prevents illegitimate visits from insects (Ojeda *et al.*, 2013).

As an strategy to understand the adaptation to particular pollinators, it appears useful to disaggregate the pollination syndromes into individual (Dell'Olivo & Kuhlemeier, 2013). In this paper, floral traits involved in attraction cues and reward were analyzed along with the physical interaction of flower with visitors (petal epidermal surface) in *Navaea phoenicea* (Vent.) Webb & Berthel., a Canarian relict btaxon belonging to the Malvaceae family. As a member of the MBE, it shows the archetypical floral traits such as a big sized, red-salmon corollas, large amounts of dilute nectar and the presence of revolute auricles at the basis of the petals conforming a conspicuous nectar receptacle. However, during the assessment of plant-pollinator interactions we identified a high frequency of visits of non-legitimate insect species together with generalist passerines. Particularly, we observed nonnative bees (*Apis mellifera*) and native species (*Bombus canariensis*, *Pararge xiphioides* and *Ancistrocerus haematodes*, see chapter 4), challenging the insect-deterrence hypothesis for red corollas. To provide an explanation to insect visits, first we aimed to provide an analytical study of the color properties of *N. phoenicea* and how are they perceived by flower visitors, within the natural context where the species lives. The questions here were: 1) what are the color properties of *N. phoenicea* flowers and their variation? 2) are there any relevant color differences between different parts of the flower? and 3) how easily are flowers perceived and detected by bees, bumblebees and blue tits within the natural habitat and how distinct are from other co-flowering species? Second, we studied nectar production dynamics and sugar production to address these particular questions: 1) what are the nectar production dynamics and its variability in space and time? What are the effects of nectar extraction and the implications for nectar concentrations? and 2) how variable is nectar sugar composition between individuals and populations?. Finally, we characterized the petal epidermal surface across the entire surface of the flower. The particular questions were: 1) is the flower of *N. phoenicea* characterized by a single epidermal type across the entire flowers? and 2) is there evidence of a petal micromorphology that favors insect interaction?

MATERIALS & METHODS

Study species

Navaea phoenicea is a threatened big sized shrub endemic to the island of Tenerife. The distribution is narrow and restricted to the northern cliffs of the oldest volcanic cores of the island, the mountain massifs of Teno (West) and Anaga (East). Its habitat is mainly the transition

Table 1. Electrostimulation values obtained for each photoreceptor in bumblebees, bees and blue tits. Lower parts correspond to the average of reflectance measures for each of them. The overall values for external and internal sides of petals correspond to the average of all the reflectance measures across the side of the petal.

	Apis mellifera			*Bombus terrestris dalmatinus*			*Cyanistes caeruleus*			
	Eu	**Eb**	**Eg**	**Eu**	**Eb**	**Eg**	**Eu**	**Es**	**Em**	**El**
Abaxial bottom	0.68	0.59	0.49	0.70	0.61	0.49	0.71	0.57	0.43	0.64
Abaxial middle	0.74	0.59	0.51	0.76	0.62	0.50	0.75	0.56	0.45	0.68
Abaxial upper	0.75	0.57	0.49	0.76	0.60	0.48	0.74	0.53	0.43	0.69
Adaxial bottom	0.71	0.67	0.57	0.74	0.68	0.57	0.76	0.65	0.53	0.64
Adaxial middle	0.78	0.60	0.53	0.79	0.63	0.52	0.77	0.57	0.47	0.71
Adaxial upper	0.75	0.56	0.50	0.76	0.59	0.48	0.74	0.53	0.43	0.69
Ventral average	0.75	0.61	0.53	0.77	0.63	0.52	0.76	0.58	0.47	0.68
Dorsal average	0.75	0.61	0.53	0.77	0.63	0.52	0.76	0.58	0.47	0.68
Staminal column	0.52	0.48	0.35	0.52	0.50	0.35	0.52	0.47	0.28	0.58
Receptacle	0.41	0.43	0.34	0.43	0.45	0.34	0.49	0.42	0.29	0.50

between sclerophilous shrubland and humid laurel forest. Field sampling was conducted in the subpopulations of Chinamada (28.562ºN, 16.290º W), Batanes (28.549ºN, 16.296º W), Taborno (28.557ºN, 16.264 ºW) and Tope Carnero (28.544ºN, -16.317º W) in Anaga massif; San Juan del Reparo (28.366ºN, -16.759ºW), Cecilia (28.367ºN, 16.830 W), Cuevas Negras (28.352º N, 16.811ºW) and Teno Alto (28.343ºN, 16.876ºW) in Teno massif. *N. phoenicea* flowers synchronically from September to March peaking at the beginning of December. First rains after the summer drought seem to trigger the flowering period.

Analysis of flower color perception

We measured the reflectance spectra of leaves for a large number of the species within the habitat of *Navaea phoenicea*, to be used as background for subsequent color analyses. We sampled three flowers per individual in five individuals across the eight localities together with all 37 co-flowering species and 79 spectra of leaves, between 3 and 5 flowers per species. For our study species, we measured different parts of the corolla, including upper, medium and lower areas of the petal, dorsal and ventral sides, auricles conforming the nectar receptacle and the staminal column (figure 1). We used an Ocean Optics USB-2000 spectrometer and a Top Sensor System Deuterium-Halogen DH-2000 lamp as a standardized

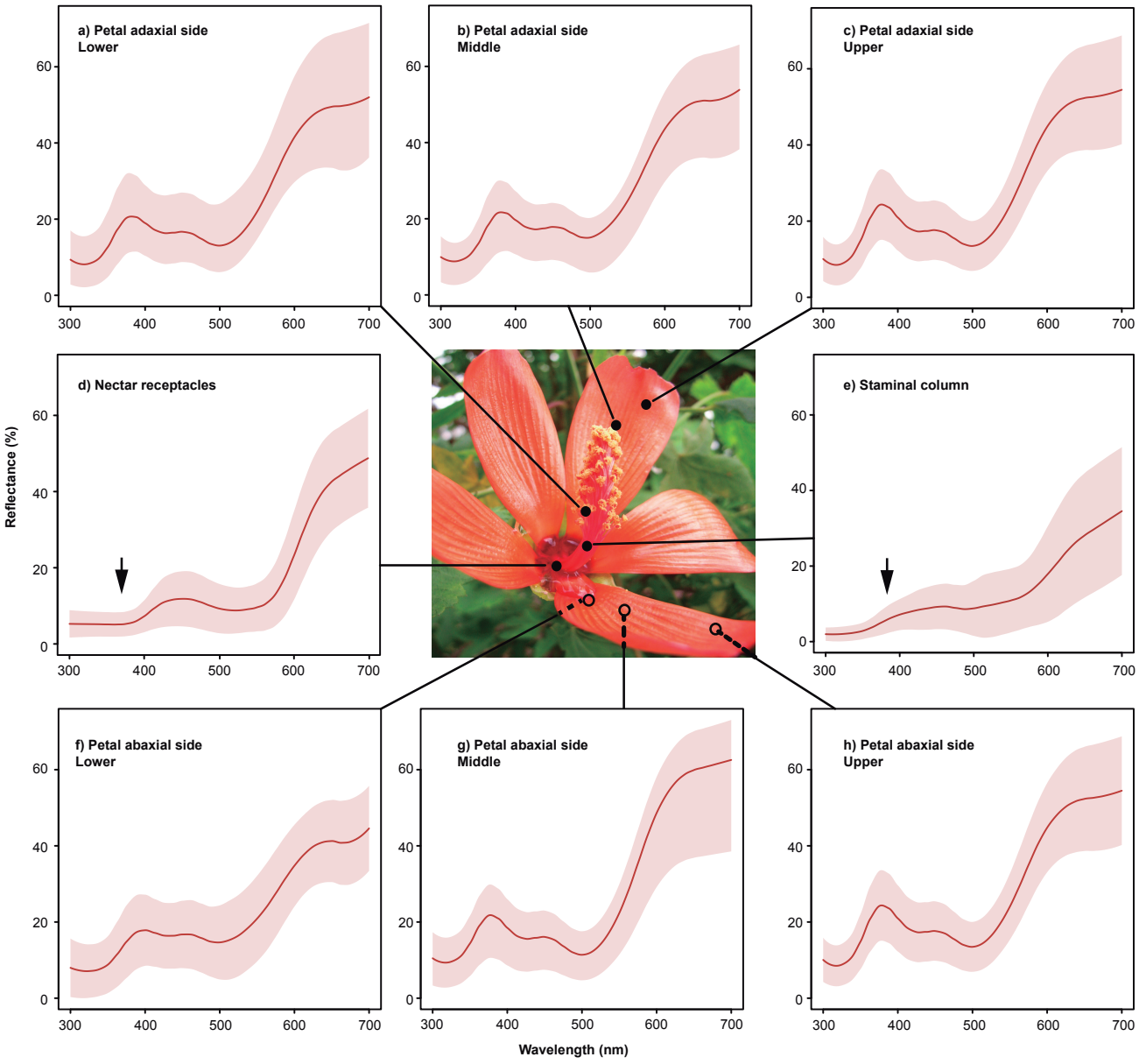


Figure 1. Floral reflectance in *Navaea phoenicea* . Averages of spectral curves measured for each flower part. Shaded zone indicate 95% confidence intervals. Arrows indicate missing UV peaks that are present in other spectral records.

light source (DT-MINI-GS-2) along a coaxial fiber (QR-400-7-UV-VIS-BX; Ocean Optics). Reflectance was measured as the proportion of a standard white reference tile (WS-1-SS; Ocean Optics, Duiven, The Netherlands) with an angle of illumination and reflectance of 45º. Spectra data were processed with Spectra Suite (Ocean Optics, 2009) and subsequent spectral calculations were performed with R software (R Core Team, 2013). Because raw spectral data were given every 0.3 nm of wavelength, all obtained spectra were interpolated to 1 nm with *pavo* package (Maia *et al.*, 2013). Bees and bumblebees visual systems are substantially different from avian systems, thus visual models for both groups were performed separately. The intensity of the electric excitations for insect photoreceptors were calculated following equations described in Chittka (1992). The mean of the measured leaves was taken as the background variable, and D65 standard measure as the illuminant. Calculations were performed for *Apis mellifera* ultraviolet (UV), blue and green photoreceptors. In the absence of data for *Bombus canariensis* we selected *B. terrestris* subsp. *dalmatinus* photoreceptor sensitivity data (maximum sensitivities at UV λmax=348 nm, blue λmax=435 nm, green λmax=533 nm; Skorupski *et al.*, 2007). For each species, the excitation values were plotted in the hexagonal chromaticity diagram (Chittka, 1992). Measured leaves were also included to determine the discriminant ability of flowers from the background. For birds, color vision was modelled with *pavo* package in a tridimensional color space. Here, *Cyanistes caeruleus* visual system was chosen (UV λmax=374 nm, blue λmax=455 nm, green λmax=539 nm, and red λmax=607 nm; Hart, 2001). We assumed that *C. caeruleus* is representative of all passerines foraging on nectar as there is little variation between species with respect to the sensitivity of photopigments (Hart, 2001).

Table 2. a) Effects of receptacle position on nectar volume, concentration and sugar yield; b) Effects of removal rate, year and flowering stage on nectar volume, concentration and sugar yield.

a) Effects of receptacle position			
	df	**F**	**P**
Concentration	4	3.083	0.0152
Volume	4	5.624	0.000166
Sugar amount	4	6.432	3.78e-05
b) Effects of removal rate, flowering stage and year			
Volume			
Daily frequency of nectar extraction	2	187.594	2.2e-16
Flowering stage	4	69.511	2.2e-16
Year	2	8.593	0.00019
Sugar concentration			
Daily frequency of nectar extraction	2	3.603	0.027
Flowering stage	4	132.940	2.2e-16
Year	2	11.287	1.32e-05
Sugar amount			
Daily frequency of nectar extraction	2	63.435	2.2e-16
Flowering stage	4	64.096	2.2e-16
Year	2	11.994	6.53e-06

Nectar production and sugar concentration

Nectar measures were performed in the same four subpopulations of previous floral biology studies (chapters 3 and 4), always in late December, along three consecutive years to account for interannual variability. Floral buds were bagged with tulle mesh prior to corolla opening to avoid interferences with flower visitors. Nectar was collected with a micropipette which was able to collect liquids at 0.01 µl increment intervals. Every measure was taken separately at each receptacle to account for intrafloral variation. As flowers show slight zigomorphy due to the staminal column curvature upwards, receptacles were numbered always in the same manner with respect to the staminal column to maintain spatial order. To account for the effects of nectar removal on the production, we conducted the measures under three different frequencies of extraction: every 24, 12 and 8 hours, starting approximately at the opening of the flower. The 24 hours period was the longest timespan because nectar could overflow the receptacle capacity after one day. Sugar concentration was measured with a hand refractometer. Moist and temperature conditions were registered in every population along the whole flower lifespan. As subpopulations are composed by fairly aggregated individuals, we considered the same conditions for all the individuals. We measured between three and five flowers per individual for every frequency of extraction, five individuals per subpopulation.

Effects of year, population, climate conditions, development stage of the flower and nectar receptacles over nectar production rates and sugar concentration were tested by means of generalized linear mixed models with REML and assuming a Gamma distribution with identity link function, as implemented in *lme4* package in R (Bates *et al.*, 2013), with plant identity as random factor. Post hoc comparisons were performed after Bonferroni correction with the *multcomp* package (Hothorn *et al.*, 2008).

Variation in nectar sugar composition

We collected nectar from five flowers per plant in 3-8 plants across all the populations at the end of the nectar sampling period. Five µl per nectary were extracted and rinsed into a 3 mm Whatman paper. Samples were stored in silica gel and kept at -20ºC until processed. Nectar-containing wicks were individually placed into 2-mL Eppendorf tubes, and 500 µl of HPLC grade water was added to each one. Each sample was measured independently three times. For each measurement, 10 µL of solution was injected into a Dionex DX 500 HPLC system (Dionex, Sunnyvale, California, USA). Retention times were calibrated daily for D-glucose, D-fructose, and sucrose (Sigma-Aldrich, Madrid, Spain) by injecting 10 µl of a calibration mixture containing 5.5 ppm, 13.75 ppm, and 13.75 ppm of these sugars, respectively. The proportions of the three different sugars (glucose, fructose, sucrose) in each sample were estimated by integrating the area under the chromatogram peaks. No other sugars were detected.

We tested differences in the proportion of each of the sugars at all levels considered (plant, flower and nectary) by fitting linear mixed models with REML accounting for the three levels with the *lmer* package, with plant as a random factor. Variance component were estimated from the model. Error calculated from the repeated measures of sugar proportions within nectary was used to estimate the within-flower variance components.

Petal epidermal cell type characterization with SEM and light microscopy

Open mature flowers of *N. phoenicea* were collected from four individuals: three fresh specimens from Teno (Ravelo and Cecilia) and Anaga populations (Chinamada) and one voucher specimen (Arnoldo Santos-3153). All freshly collected specimens were preserved in 70% ethanol. The sample collected from the voucher specimen was first re-hydrated in warm water and later preserved in 70% ethanol. All four specimens were analysed with a ligth microscope Nikon Eclipse E600 and photographed using

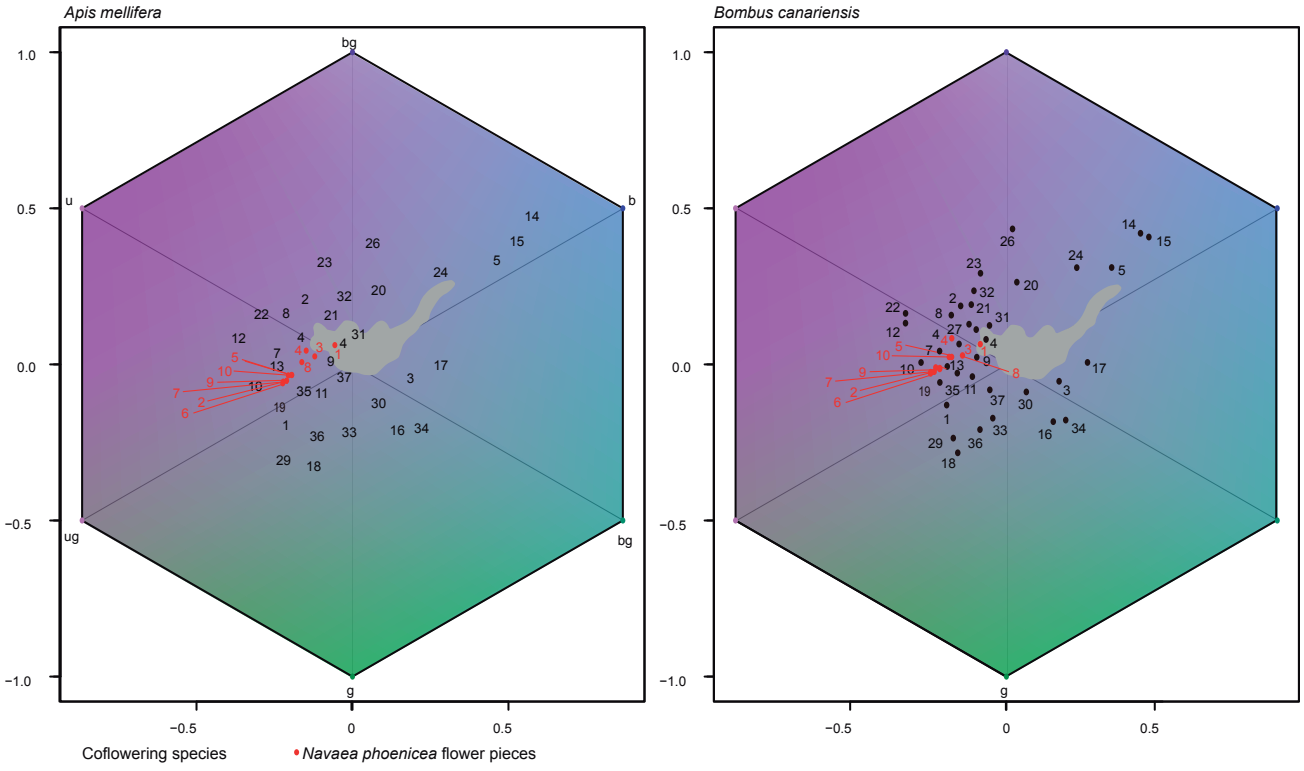


Figure 2. Hexagonal chromaticity diagram displayed for visual models based on honeybees *Apis mellifera* and bumblebees *Bombus terrestris dalmatimus* photoreceptors. Background foliage spectra are presented as a patch in grey. Coflowering species: 1 *Aeonium holochrysum*, 2 *Andryala pinnatifida*, 3 *Anthirrinum majus* (blue bottom), 4 *Anthirrinum majus* (yellow bottom), 5 *Anthirrinum majus* (lateral), 6 *Anthirrinum majus* (top), 7 *Argyranthemum frutescens*, 8 *Arisarum shimorrhinum*, 9 *Bituminaria bituminosa*,10 *Bituminaria bituminosa*,11 *Canarina canariensis*, 12 *Ceropegia dichotoma*, 13 *Echium virescens*, 14 *Eruca vesicaria*, 15 *Erysimum bicolor* (blue), 16 *Erysimum bicolor* (white), 17 *Gomphocarpus fruticosus**, 18 *Habenaria tridactylites*, 19 *Jasminum canariensis*, 20 *Justicia hyssopifolia* (top), 21 *Justicia hyssopifolia* (bottom), 22 *Justicia hyssopifolia* (lateral), 23 *Lavandula canariensis*, 24 *Limonium macrophylum*, 25 *Micromeria glomerata*, 26 *Nicotiana glauca**, 27 *Pericallis tussilaginis*, 28 *Pterocephalus dumetorus*, 29 *Rubus fruticosus*, 30 *Senecio leucanthemifolius*, 31 *Solanum vespertilio*, 32 *Solanum vespertilio*, 33 *Solanum vespertilio*, 34 *Sonchus radicatus*, 35 *Sonchus radicatus*, 36 *Teline canariensis*, 37 *Ulex europaeus**. Asterisks denote introduces species. *Navaea phoenicea* flower pieces measured: 1 Staminal column, 2 ,mean of abaxial side, 3 nectar receptacles, 4 abaxial side, bottom 5, abaxial side middle, 6 mean of adaxial side, 7 adaxial side middle, 8 adaxial side bottom, 9 adaxial side upper, 10 abaxial side upper.

an attached Nikon Digital Camera DXM 1200F. The entire surface of all petals within the flower were screened and analysed in all four specimens. Two of the specimens (Ravelo IND2, Cecilia IND1) were later analysed scanning electron microscope (SEM). These two samples were first dehydrated in a series of 80%, 90%, 95% (30 min each) and 3X in 100% (30 min each) of ethanol. The samples were later critical point dried and coated with a 12 nm layer of gold for 15 min. Portions from the lower, middle and upper part on both sides of the petal (adaxial and abaxial) were analysed and photographed at different magnifications with a Hitachi S-2600N scanning electron microscope (SEM) using an acceleration voltage of 8.0 Kv (Beam 30) at high vacuum.

RESULTS

Color analyses

The spectral reflectance of the sampled flower parts are shown in figure 1. Petals showed a first peak at 380 nm (blue). The second peak started at 500 nm and reached a maximum beyond 700 nm (red and infrared). Therefore petals reflected light not only in the red wavelength range but also in the blue and in the green range of the spectrum. There were no differences between different areas of the petal. The staminal column (figure 1e) showed the first peak at 440 nm (also blue) with lower intensity and a second peak at 700 nm but also with lower intensity

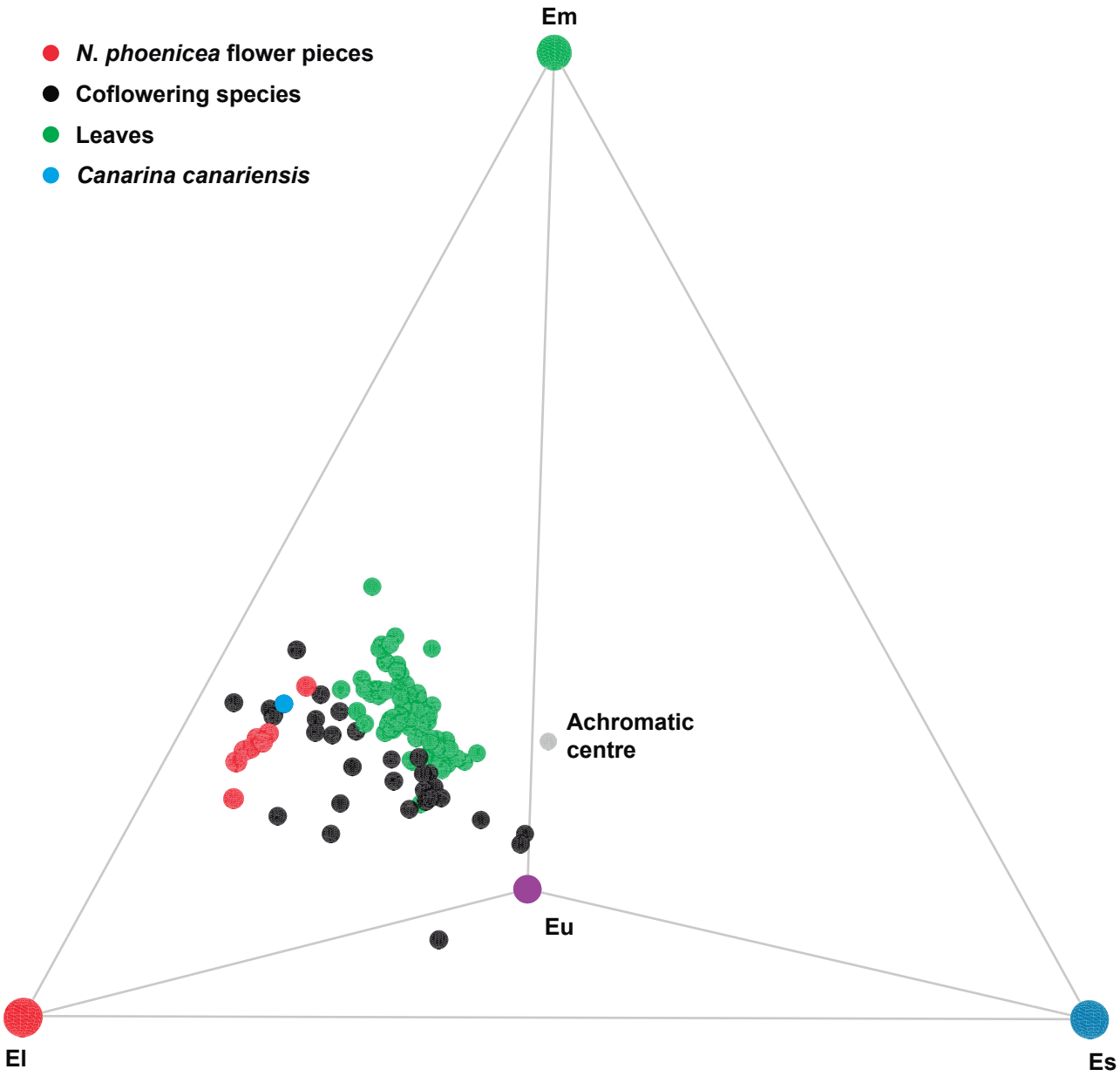


Figure 3. Tridimensional chromaticity diagram displayed for the visual model of Blue tits. Eu, Es, Em, El is the notation described by Hart (2011) for Ultraviolet, Short, Medium and Long sensitivities of avian photoreceptors.

with respect to the petals. In nectar receptacles (figure 1d) overall reflectance was low. Rather than two separate peaks the spectral curve for receptacles showed instead a gradual increase up to 700 nm, therefore with some reflectance in the yellowish range.

Photoreceptor excitation values for the three modeled species are shown in table 1. For *A. mellifera* and *B. terrestris dalmatinus*, all the petal measures reached the 0.5 threshold for detection ability against the green background. Receptacles and staminal column showed the lowest values in both insect species, with values more frequently below the threshold, therefore indicating a poor detectability against the background. Values were especially lower for the green receptor. They showed as well the lowest values among flower parts in the rest of photoreceptors. All the areas of the petals reached high values in the UV receptor and to a lower extent for the blue receptor.

For *C. teneriffae*, the UV receptor achieved higher excitation values than the red receptor. Interestingly, scores were in the UV were similar as those for insects. Green receptor had lower excitation values, which were especially low for receptacles and the staminal column. Therefore, both bees and bumblebees were able distinguish them from the green background. For both insect species the green photoreceptor showed the lowest values. Color hexagons drawn for *A. mellifera* and *B. terrestris dalmatinus* (figure 2) provide graphical understanding of their discrimination ability against the background. Petals fell out of the cloud of leaves points, whereas receptacles and staminal column fell for both species at the edge, thus its visibility remained more limited For *C. caeruleus*, a clear discrimination of floral parts were in distinct areas from the cloud of leaves, and were remarkably far from it than the rest of the measured flowers (figure 3).

Nectar production

Nectar daily production ranged from 0 to 148 microliters with a mean of 10.48 microliters (median=6.00). Concentration expressed as sugar percentage ranged from 0 to 13.30% with a mean of 8.17%. Sugar amount contained in nectar ranged from 0 up to 1.8239 mg (mean=1.27 mg). The results of the tested effects on nectar properties are summarized in table 1. Nectar volume was significantly higher in receptacles 1 and 5, that is, the ones at the upper side (figure 4a and table 2a). Flowering stage was significant for nectar yield (table 2b), which reached its maximum at the anthesis stage (figure 5a). Finally, nectar volume was

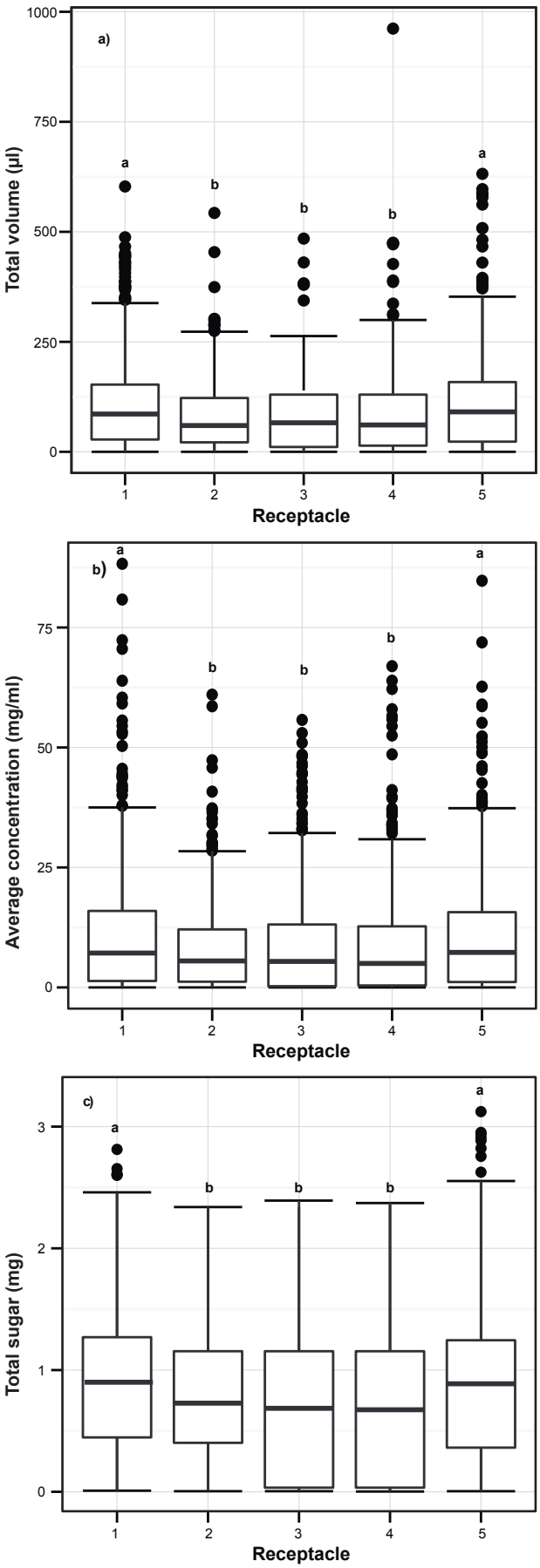


Figure 4. Boxplots for total nectar secretion along the flower lifespan, per nectar receptacle; a) volume; b) mean concentration across flower lifetime; c) total sugar yield. Groups of means statistically different are labelled with different letters.

Table 3. Summary of nectar sugar composition. Numbers are expressed as percentages of sugar proportion.

	Glucose	Fructose	Sucrose
	58.36±2.9	41.31±2.7	0.2±0.7
Variance components			
Individual	40.2	37.1	-
Flower within individual	10.4	15.5	-
Receptacle within flower	49.4	47.4	-

found to be significantly higher in 2008 than in 2006 and 2007 (figure 4c). Conversely, sugar concentration did not show significant variations (figure 5b). Sugar amount was found to be significantly higher in the upper receptacles and at the anthesis stage and in year 2008 (figure 5c).

When accounting for nectar removal, daily volume yields were 113.00±67.67 µl for 12 hours frequency and 129.52±94.34 for 8 hours extraction frequency. Therefore nectar removal twice per day almost doubled nectar production, whereas three daily removals had a lower proportional effect. Still, volume was significantly higher with three removals than with two (ANOVA, $P>0.001$). Concentration was nevertheless constant and did not diminish with higher volumes. There were also significant differences between years (table 2b and figure 5).

Nectar sugar composition

The proportion of detected sugars in nectar are presented in table 3. Sugar proportions were almost invariably 58% glucose and 41% fructose, with only traces of sucrose in a few individual measurements (figure 6). Glucose was significantly prevalent over fructose ($t=63.54$, $df=608.386$, $P=2.2\text{-}10^{-6}$). The analysis of components of variance showed little variation in the sugar proportion, which is mostly attributed to intrafloral and among individuals levels (table 3). The lowest percentage of variance was attributed to flowers even when they were measured at different stages, hence analysis did not detect a significant effect of stage on the sugar composition. Glucose significantly increased with concentration whereas fructose decreased proportionally (GLU: $t=15.45$, $df=327$, $P=2.2\text{-}10^{-6}$; FRU: $t=16.72$, $df=327$, $P=2.2\text{-}10^{-6}$).

Anatomy of epidermal cells

The entire petal surface showed tabular rugose cells with vertical striations (TRS). The cells located at the base of the petals were less differentiated (cells of simple shape, without prominent surface features and characteristic of early developmental stages) while cells located at the upper side of the petal (tip of the petal) were more differentiated. Overall, the adaxial side (exposed to pollinators) is more differentiated than the abaxial side of the petal. We did not find the presence of trichomes or papillate cells in the surface of the five petals analysed in the four specimens (figure 7).

DISCUSSION

In this paper we provide the first integrative, detailed study of ornithophilous floral traits in a Canarian ornithophilous species. The assemble of flower characters can provide new insights on the evolution of reproductive

Table 4. Summary of epidermic cell types and known nectar sugar composition for Malveae species.

Species			Nectar composition	Phylogeny	Pollinator type
*Navaea phoenicea**	TR	TR	FG, Fdez de Castro 2015		Opportunistic birds
Lavatera acerifolia			FG, Dupont *et al.* 2004		
Lavatera thuringiaca	PO[t]	PO			
Malva moschata	TR[t]	TR[t]	sFG , Percival 1961	Lavateroid clade	
Lavatera olbia	PO	PO[t]	SFG, Percival 1961		
Lavatera trimestris	PO[t]	PO[t]	SFG, Percival 1961		
Lavatera triloba	PO[t]	PO[t]			
Malva neglecta	PO[t]	PO[t]			
Malva sylvestris	TR[t]	TR[t]	FG, Percival 1961	Malvoid clade	Insects
Lavatera arborea	PO[t]	PO[t]	SFG, Percival 1961		
Lavatera maritima	PO[t]	PO[t]			
Alcea rosea	PO[t]	PO[t]	sFG, Percival 1961		
Alcea pallida	PO[t]	PO[t]		Alcea clade	
Althaea officinalis	PO[t]	PO[t]			
Malope trifida	PO[t]	PO[t]			

strategies in the MBE. Our results in *Navaea phoenicea* suggest that only strategies for bird attraction are involved, which does not deter illegitimate nectar robbers. We therefore show an inconsistent set of floral traits, because adaptations in visual cues, reward, and mechanic barrier have different consequences on visitor behaviour. Although *Navaea phoenicea* is considered a legitimate MBE species, color and nectar are in fact similarly attractive for passerine birds and for non-pollinator insects, given their frequency of visits (chapter 4), while structural adaptations providing exploitation barriers are not efficient to exclude illegitimate visitors.

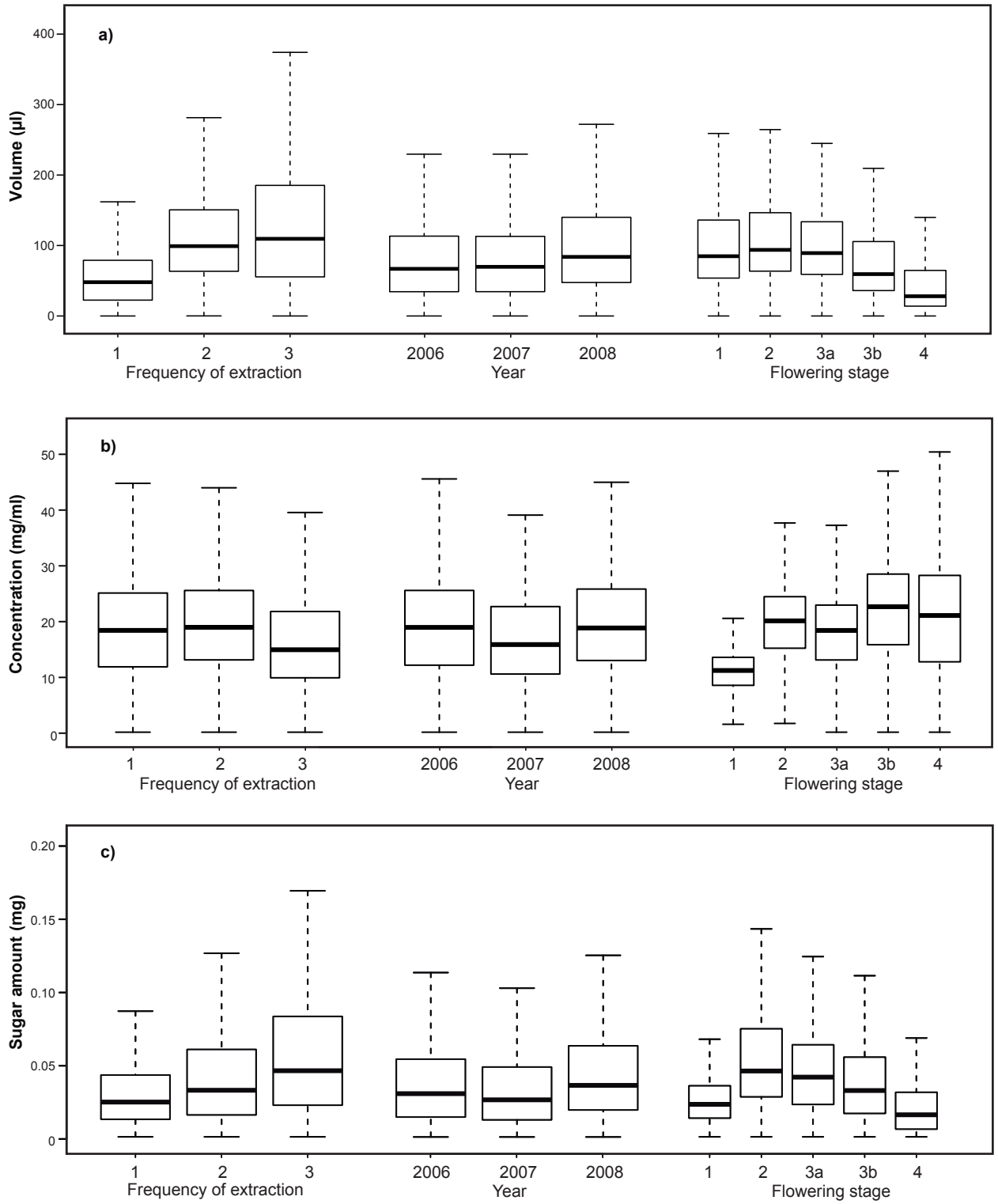


Figure 5. Boxplots for nectar volume, sugar concentration and sugar yield grouped by: daily frequency of extraction (1 per day, 2 per day every 12 hours and 3 per day every 8 hours), year of sampling and flowering stage; 1= open flowers, closed anthers; 2 = anthesis stage (male stage); 3a = exerted erect styles; 3b = exerted recurved styles; 4= senescent flowers.

Attraction by color is more than reddish corollas

We did not find any spatial color pattern serving as a guide for pollinators in the flowers. We did not detect significant differences between petal parts, nor post-anthesis changes in flower color. The nectar receptacles are the most structurally unique part for which color distinctiveness could be expected, but they were instead the less detectable flower pieces considering a green foliage. As petals would be actually the true background, they would remain much more cryptic indeed. Beyond the subjective perception of flower color by the human eye, the examination of reflectance data sheds light to the role of color in plant-pollinator interactions. Our spectral analysis uncovers a secondary color signal at the UV-Blue wavelength, which therefore places the species flowers not in the green locus as expected but in the ultraviolet instead. The UV-blue reflectance implies that flowers are better detected by hymenopterans than a priori expected based solely on human perception, which partially explains the high frequency of visits by insects. Two other factors can contribute to a higher visitation frequency. These factors rely on the fact that even in the absence of a stronger spectral signal, red colors may be still detected by hymenopterans provided the background allows enough contrast. The first factor relates to long and predictable flowering season of *Navaea phoenicea*, where individuals have an aggregated distribution in the subpopulations (chapter 1), providing an expectable, constant spot of energy and water for any visitors, which can be determinant for both insects and birds based on their learning abilities (e.g. Chittka and Raine, 2006). The second factor relates to the putative higher ability of island bumblebees to detect red colors as a result of higher sensitivity of their green receptors to red wavelengths (Chittka *et al.*, 2004; Skorupski *et al.*, 2007). This mechanism would account for *Bombus canariensis*, but not the introduced honeybee.

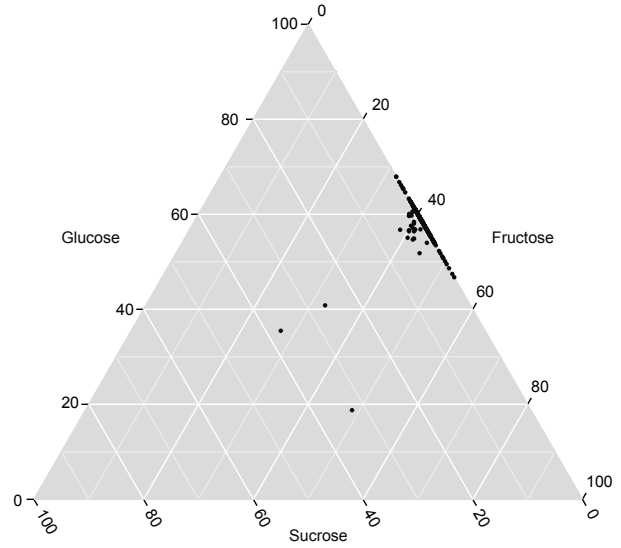


Figure 6. Sugar composition: Ternary diagram displaying the proportions of the three sugars. Each point represent the average value of the repeated measures conducted for each sample.

The maintenance of visual cues for insects brings two implications for the discussion of color evolution. The first would relate to the evolution of flower pigmentation of *Navaea phoenicea* with respect to the close Malva clade. The same UV-blue peak is persistently constant across the tribe, according the available spectra submitted to the FrED reflectance database (Arnold *et al.*, 2010). This may denote the existence of the same anthocyanin pigment type across Malveae species, synthetized through the same pathway, whereas the red pigmentation may be explained either by: 1) as a shift in one of the biosynthetic pathways of flavonoids to produce, for instance any red-colored anthocyanins (pelargonidin-based or cyanidin-based); 2) with a change in vacuolar pH (Cronk & Ojeda, 2008). This hypothesis is unlikely considering that the double peak indicates more than one pigment, or 3) cell shape, as conical cells typically present in the petals can influence on how the light is reflected by cells as documented in *Lotus* (Ojeda *et al.*, 2012). Anthocyanins have been widely characterized across a good number of species of the Malveae tribe (Takeda *et al.*, 1989; Harborne *et al.*, 1994; Farina *et al.*, 1995), but mostly blue pigments, rather than red ones. On the contrary, pigments in genera *Hibiscus* and *Gossypium* are better characterized, mainly for commercial purposes (e.g. Feng *et al.*, 2014). The evolution of regulatory genes of anthocyanin pathways is poorly understood in the family, with only one gene (chalcone synthase) involved on the early steps of the anthocyanin pathway characterized in several species of the family (Zhou *et al.*, 2011).

The second insight relates to the selective pressures on flower color. Previous works shows that at least three ornithophilous taxa are well detected by birds and poorly discriminated by bumblebees or bees as consequence of exclusive long-wave reflectance (Ollerton *et al.*, 2009). This work in turn shows that petals of *N. phoenicea* are not cryptic for insects, due to a UV-Blue reflectance peak. The role of the red color has been discussed not only as a bird attractor but as a deterrence mechanism for illegitimate bee visitors which may harm reproductive fitness through nectar robbing or pollen limitation (e.g. Rodríguez-Gironés and Santamaría, 2004). For instance, the introduction of honeybees in the habitat of the ornithophilous *Echium*

wildprettii subsp. *wildprettii* drives nectar depletion of flowers and prevents legitimate visits of chiffchaffs (Valido *et al.*, 2002, 2014). The insect exclusion hypothesis does not hold, as hymenopteran discrimination ability may be high enough to select red flowers as mentioned above. Our work suggests that color in *N. phoenicea* evolved to attract birds, without aiming to exclude illegitimate visitors. This was unexpected, as color is considered a labile trait which can shift rapidly in response to selective pressures (Schiestl & Johnson, 2013). Considering the high nectar amounts secreted by the plant and the effect of nectar removal, it does not appear that nectar robbing by insects has a major negative effect on fitness. Moreover, there is not pollen discount driven by insects, as pollen removal was not observed (chapter 4). Hence, selective pressures to deter nectar robbers does not exist.

Finally, these results can be related to the hypothesis of a greater abundance of red flowers with higher nectar rewards in islands (Skorupski *et al.*, 2007), driving an innate preference of bumblebees for red flowers. The existence of the MBE provides some support for such abundance, especially as several species provide the nectar during the winter season, but to date the hypothesis remains untested. This study provides additional evidence for it.

Nectar production as an adaptation to bird pollination

Nectar production was remarkably high along all flowering stages. In this study we managed to assess significant factors that affect nectar production, and confirmed the stability in nectar sugar composition. Along all the flowering period, a flower produces 349 µl of nectar on average and 4.55 mg of sugars without removal. On average, one mature individual produces 176 flowers along a single season (chapter 6) which leads to a final expenditure of 60 ml of water and 76 joules without considering any energy conversion discount.

Nectar dynamics and variability show several insights around both the evolution of the bird-flower syndrome and the mating system of *N. phoenicea*. First, the asymmetric nectar production found between upper and lateral/lower petal receptacles confers a zygomorphic condition in the same manner that results from the upwards curvature of the staminal column as whole, and of anthers individually (figure 1). This selection of floral shapes that lead to a divergence of the radial symmetry has been frequently reported (e.g., Gómez *et al.*, 2014, 2006). Likewise several species without remarkable bilateral symmetry have been observed to provide different nectar yields between nectaries (Biernaskie *et al.*, 2004; Anand *et al.*, 2007). An interpretation of those facts is that spatial asymmetry

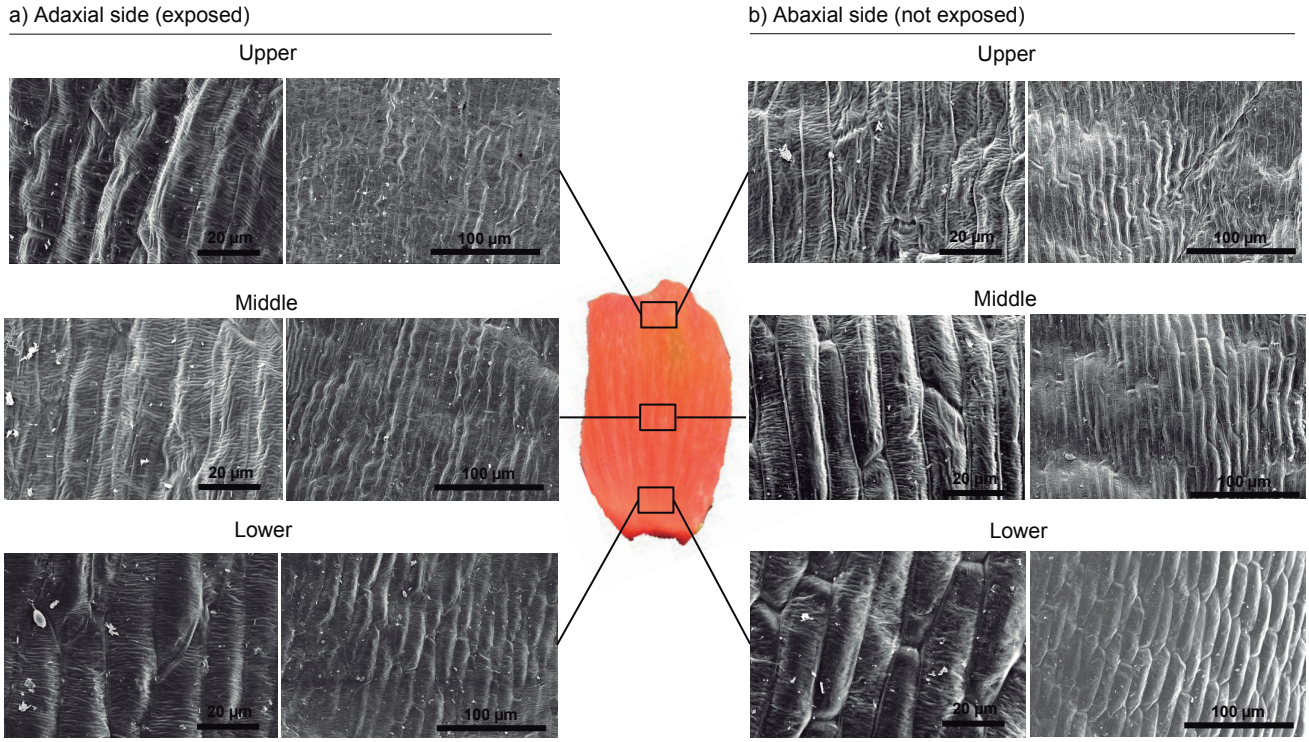


Figure 7. . Petal micromorphology on the petal surface of *Navaea phoenicea*. The same epidermal type, tabular rugose cells, without the presence of trichomes or papillate cells was observed in the adaxial side (a) and abaxial side (b) of the petal.

of rewards within the flower has appeared to optimize pollinator accuracy (Armbruster *et al.*, 2009) of perching pollinators, similar to that of African sunbirds, and probably Canarian warblers. Furthermore, floral orientation appears to optimize perching habits as well (Fenster *et al.*, 2009). On the other hand, the most effective pollinator among visitors is *P. canariensis*, which hovers from below more frequently than perch when foraging, (chapter 5) resulting in limited seed sets. At the plant community level – the thermophilous shrubland in autumn-winter season – this zygomorphic pattern shows a greater specialization on pollinators which do not appear to be able to forage in any other co-flowering plant (personal observation). Therefore nectar secretion seems to be a consequence of pollinator foraging behavior and resource partitioning (Rodríguez-Gironés & Santamaría, 2010).

The second significant effect is the reward effort allocated across flowering stages. The higher volume detected at the anthesis stage does not necessarily indicate a stronger effort towards the male function because this stage lasts only 24 hours, as pollen is rapidly shed. The female stage in turn lasts longer (chapter 3). Protandry is interpreted in a number of mallows as a mechanism to separate male and female functions but with similar amounts of resources allocated for both functions (Ruan *et al.*, 2010). This is in line with the general trend of a balanced allocation between sexes when visitation rates are high (Muchhala *et al.*, 2010). The greater volumes at the male stage may compensate the shorter anthesis time with respect to style appearance once the pollen is shed by maximizing the attraction of pollinators.

Finally, the third effect relates to volume variance across the years. We observed significant differences in nectar yields between years. The variation across years of nectar production is important, as differences in climate variables such as rainfall can influence the amount of available water. As the flowering season appears to be triggered by the first rains after the drought summer period, a relative water limitation may act as one of the factors conditioning the reproductive effort. Despite the massive secretion of diluted nectar, water availability can modulate the production given that moister years may favor even higher volumes.

Nectar sugar composition formed almost exclusively by monosaccharides was strongly constant across samples, and results were consistent with the sample analyzed by Dupont *et al.* (2004) and other studies (Johnson & Nicolson, 2008). The constant prevalence of glucose against fructose denotes that sucrose is hydrolyzed by cell-wall invertase and monosaccharides are secreted afterwards by different sugar transporters (Williams *et al.*, 2000) leading to different sugar proportions. The implications of sugar composition in MBE species have been discussed elsewhere (Dupont *et al.*, 2004a), but we stress again that the basis of the association of sugar types with pollinator syndrome is, at present, based on weak or even contradictory evidence (Lotz & Schondube, 2006). Without testing the actual capacity and preferences of sugar types in passerines there is little to interpret about shifts in this fundamental trait. In the context of the evolution of mallows, there is as well weak evidence how evolution of nectar sugar composition proceeds. The information for mallow species which is available in the literature is scarce (Percival 1961, table 4) but it does not indicate the existence of any differences between lineages. In most of the species, all enthomophilous, sucrose is present at different levels, with only *Malva sylvestris* matching both nectar properties and epidermal cell types of *Navaea phoenicea*. Consequently, a better understanding of the evolution of floral traits in the Malvaceae is needed.

Ecological implications of epidermal cells type

Insects visit *N. phoenicea* always in the same manner (personal observation). Lower petals can be used sparely as a landing platform to approach nectar receptacles as the horizontal floral display can eventually help insects to grip. The basis of the staminal column is more frequently reached by insects to get some stability, but insects do not get in contact with anthers or exerted stigmas. Therefore epidermal surface of petals does not completely deter access of illegitimate visitors to the nectar receptacles.

Rewards and visual traits in *Navaea* flowers are attractive for both birds and insects. There is clear evidence that these cues respond to an adaptation to bird pollination, but at the same time they do not prevent nectar robbers to visit the flowers. Accounting for epidermal cell types allows a more accurate interpretation, and leads to two different hypotheses. On the one hand the loss of conical cells may constitute a barrier big enough to deter a higher amount of illegitimate visitors. Given that the frequency of insect visits is still very high, this hypothesis does not hold. The second hypotheses would postulate that the massive amount of nectar secreted is a consequence of resource

redirection for perching birds. Therefore, nectar robbing would not interfere with pollinators foraging on the flowers, and therefore there would not exist any selective pressure for the suppression of the biosynthetic pathway of the UV-blue signals. The extent of the interference of *A. mellifera* on reproductive success needs nevertheless to be examined (Dupont *et al.*, 2004; Valido *et al.*, 2014).

An additional consideration is that, in the pollination landscape of the thermophilous shrub habitat in the winter, *Navaea phoenicea* flower traits are fairly unique. Winter flowering could be selected either for resource availability but also to optimize the visitation rate in the absence of other energy resource on one side, but also to avoid the higher period of insect activity, as lower temperatures lead to a decrease in insect visitation rates (chapter 4).

In this paper we showed that the convergence of floral traits driving a pollination syndrome does not necessarily imply the integration of the ensemble of floral traits. In turn, selection pressures act here on those traits that assure not only reproductive success to the species, but also which neutralize the selection pressures exerted of antagonist mutualisms. As a concluding remark, we highlight that several features of the reproductive biology of *N. phoenicea* need to be analysed: characterizing nectary anatomy, amino acid composition and the phylogenetic approaches to flower pigments in mallows and the evolution of petal epidermal cells can contribute to an accurate disentanglement of interactions of the MBE with birds and insects.

REFERENCES

Anand C., Umranikar C., Shintre P., Damle A., Kale J., Joshi J., & Watve M. (2007) Presence of two types of flowers with respect to nectar sugar in two gregariously flowering species. *Journal of Biosciences*, **32**, 769–774.

Armbruster W.S., Hansen T.F., Pélabon C., Pérez-Barrales R., & Maad J. (2009) The adaptive accuracy of flowers: measurement and microevolutionary patterns. *Annals of Botany*, **103**, 1529–45.

Arnold S.E.J., Faruq S., Savolainen V., McOwan P.W., & Chittka L. (2010) FReD: the floral reflectance database: a web portal for analyses of flower colour. *PloS One*, **5**, .

Bates D., Maechler M., & Bolker B. (2013) lme4: Linear mixed-effects models using S4 classes. Version 0.9.

Bergamo P.J., Rech A.R., Brito V.L.G., & Sazima M. (2015) Flower colour and visitation rates of *Costus arabicus* support the "bee avoidance" hypothesis for red-reflecting hummingbird-pollinated flowers. *Functional Ecology*, in press.

Biernaskie J.M., Cartar R. V., Sciences B., & Tk C. (2004) Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Functional Ecology*, **18**, 125–129.

Chittka L. (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A*, **170**, 533–543.

Chittka L., Ings T.C., & Raine N.E. (2004) Chance and adaptation in the evolution of island bumblebee behaviour. *Population Ecology*, **46**, 243–251.

Chittka L. & Raine N.E. (2006) Recognition of flowers by pollinators. *Current opinion in Plant Biology*, **9**, 428–435.

Cronk Q. & Ojeda D.I. (2008) Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, **59**, 715–727.

Dell'Olivo A. & Kuhlemeier C. (2013) Asymmetric effects of loss and gain of a floral trait on pollinator preference. *Evolution*, **67**, 3023–31.

Dupont Y.L., Hansen D.M., Rasmussen J.T., & Olesen J.M. (2004a) Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. *Functional Ecology*, **18**, 670–676.

Dupont Y.L., Hansen D.M., Valido A., & Olesen J.M. (2004b) Impact of introduced honey bees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biological Conservation*, **118**, 301–311.

Faegri K. & Van Der Pijl L. (1979) *The principles of pollination ecology*. Elsevier, Oxford.

Farina A., Doldo A., Cotichini V., Rajevic M., Quaglia M.G., Mulinacci N., & Vincieri F.F. (1995) HPTLC and reflectance mode densitometry of anthocyanins in *Malva sylvestris* L: A comparison with gradient-elution reversed-phase HPLC. *Journal of Pharmaceutical and Biomedical Analysis*, **14**, 203–211.

Feng H., Li Y., Wang S., Zhang L., Liu Y., Xue F., Sun Y., Wang Y., & Sun J. (2014) Molecular analysis of proanthocyanidins related to pigmentation in brown cotton fibre (*Gossypium hirsutum* L.). *Journal of Experimental Botany*, **65**, 5759–5769.

Fenster C.B., Armbruster W.S., & Dudash M. (2009)

Specialization of flowers: is floral orientation an overlooked first step? *New Phytologist*, **183**, 502–506.

Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R., & Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 375–403.

Gómez J.M., Perfectti F., & Camacho J.P.M. (2006) Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *The American Naturalist*, **168**, 531–45.

Gómez J.M., Perfectti F., & Klingenberg C.P. (2014) The role of pollinator diversity in the evolution of corolla-shape integration in a pollination-generalist plant clade. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **369**, 20130257.

Harborne J.B., Saito N., & Detoni C.H. (1994) Anthocyanins of *Cephaelis*, *Cynomorium*, *Euterpe*, *Lavatera* and *Pinanga*. *Biochemical Systematics and Ecology*, **22**, 835–836.

Hart N.S. (2001) The visual ecology of avian photoreceptors. *Progress in Retinal and Eye Research*, **20**, 675–703.

Hothorn T., Bretz F., & Westfall P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Johnson S.D. & Nicolson S.W. (2008) Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters*, **4**, 49–52.

Lotz C.N. & Schondube J.E. (2006) Sugar Preferences in Nectar-and Fruit-Eating Birds: Behavioral Patterns and Physiological Causes. *Biotropica*, **38**, 3–15.

Maia R., Eliason C.M., Bitton P.P., Doucet S.M., & Shawkey M.D. (2013) pavo: An R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution*, **4**, 906–913.

Muchhala N., Brown Z., Armbruster W.S., & Potts M.D. (2010) Competition drives specialization in pollination systems through costs to male fitness. *The American Naturalist*, **176**, 732–743.

Nicolson S.W. (2002) Pollination by passerine birds: why are the nectars so dilute? *Comparative biochemistry and physiology.Part B, Biochemistry & molecular biology*, **131**, 645–652.

Nicolson S.W. & Fleming P. a (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. *Plant Systematics and Evolution*, **238**, 139–153.

Van der Niet T. & Johnson S.D. (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology and Evolution*, **27**, 353–361.

Van der Niet T., Peakall R., & Johnson S.D. (2014) Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany*, **113**, 199–211.

Ocean Optics (2009) Spectra Suite 1.0.

Ojeda D.I. (2013) The Macaronesian bird-flowered element as a model system to study the evolution of ornithophilous floral traits. *Vieraea*, **41**, 73–89.

Ojeda D.I., Santos-Guerra A., Caujapé-Castells J., Jaén-Molina R., Marrero-Rodríguez Á., & Cronk Q. (2012) Comparative micromorphology of petals in Macaronesian *Lotus* (Leguminosae) reveals a loss of papillose conical cells during the evolution of bird pollination. *International Journal of Plant Sciences*, **173**, 365–374.

Ojeda D.I., Santos-Guerra A., Oliva-Tejera F., Valido A., Xue X.., Marrero-Rodríguez Á., Caujapé-Castells J., & Cronk Q. (2013) Bird-pollinated Macaronesian *Lotus* (Leguminosae) evolved within a group of entomophilous ancestors with post-anthesis flower color change. *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 193–204.

Olesen J.M. & Valido A. (2004) Lizards and birds as generalized pollinators and seed dispersers of island plants. *Insular Ecology* (ed. by J.M. Fernández Palacios and C. Morici), pp. 229–250. Asociación Española de Ecología Terrestre y Excmo.Cabildo Insular de La Palma, La Palma.

Ollerton J. (1998) Sunbird surprise for syndromes. *Nature*, **394**, 726–227.

Ollerton J., Cranmer L., Stelzer R.J., Sullivan S., & Chittka L. (2009) Bird pollination of Canary Island endemic plants. *Die Naturwissenschaften*, **96**, 221–32.

R Core Team (2013) R: A Language and environment for statistical computing. Version 3.1.2.

Rodriguez-Gironés M. a. & Santamaria L. (2010) How foraging behaviour and resource partitioning can drive the evolution of flowers and the structure of pollination networks. *Open Ecology Journal*, **3**, 1–11.

Rodríguez-Gironés M. a. & Santamaría L. (2004) Why are so many bird flowers red? *PLoS Biology*, **2**, 1515–1519.

Rodríguez-Rodríguez M.C., Jordano P., & Valido A. (2013) Quantity and quality components of effectiveness in insular pollinator assemblages. *Oecologia*, **173**, 179–90.

Rodríguez-Rodríguez M.C. & Valido A. (2011) Consequences of plant-pollinator and floral-herbivore interactions on the reproductive success of the Canary Islands endemic *Canarina canariensis* (Campanulaceae).

American Journal of Botany, **98**, 1465–1474.

Rosas-Guerrero V., Aguilar R., Martín-Rodríguez S., Ashworth L., Lopezaraiza-Mikel M., Bastida J.M., Quesada M., & Martén-Rodríguez S. (2014) A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters*, **17**, 388–400.

Ruan C.-J.J., da Silva J.A.T.., Qin P.P.., & Teixeira da Silva J. a. (2010) Style curvature and its adaptive significance in the Malvaceae. *Plant Systematics and Evolution*, **288**, 13–23.

Schiestl F.P. & Johnson S.D. (2013) Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution*, **28**, 307–315.

Shrestha M., Dyer A.G., Boyd-Gerny S., Wong B.B.M., & Burd M. (2013) Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist*, **198**, 301–310.

Skorupski P., Döring T.F., & Chittka L. (2007) Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology*, **193**, 485–94.

Takeda K., Enoki S., Harborne J.B., & Eagles J. (1989) Malonated Anthocyanins in Malvaceae - Malonylmalvin from Malva sylvestrfis. *Phytochemistry*, **28**, 499–500.

Trøjelsgaard K., Báez M., Espadaler X., Nogales M., Oromí P., Roche F. La, & Olesen J.M. (2013) Island biogeography of mutualistic interaction networks. *Journal of Biogeography*, **40**, 2020–2031.

Valido A., Dupont Y.L., & Hansen D.M. (2002) Native birds and insects, and introduced honey bees visiting *Echium wildpretii* (Boraginaceae) in the Canary Islands. *Acta Oecologica*, **23**, 413–419.

Valido A., Rodríguez-Rodríguez M.C., & Jordano P. (2014) Impacto de la introducción de la abeja doméstica (*Apis mellifera*, Apidae) en el Parque Nacional del Teide (Tenerife, Islas Canarias). *Ecosistemas*, **23**, 58–66.

Valtueña F.J., Ortega-Olivencia A., & Rodriguez-Riano T. (2007) Nectar production in *Anagyris foetida* (Fabaceae): Two types of concentration in flowers with hanging droplet. *International Journal of Plant Sciences*, **168**, 627–638.

Waser N.M., Chittka L., Price M. V, Williams N.M., & Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.

Williams L.E., Lemoine R., & Sauer N. (2000) Sugar transporters in higher plants – a diversity of roles and complex regulation. *Trends in Plant Science*, **5**, 283–290.

Zhou L., Wang Y., & Peng Z. (2011) Molecular characterization and expression analysis of chalcone synthase gene during flower development in tree peony (*Paeonia suffruticosa*). *African Journal of Biotechnology*, **10**, 1275–1284.

6 Conservation and population viability of a distinct palaeoendemic species in the Canary Islands

Conservación y viabilidad poblacional de un paleoendemismo en las Islas Canarias

Conservation and population viability of a distinct palaeoendemic species in the Canary Islands

ABSTRACT

The insular biota merits a strong conservationist focus, due to the importance of islands for biodiversity and the role of key species for ecosystem processes. We monitored along ten years the populations of the shrub *Navaea phoenicea*, a flagship species endemic to Tenerife Island in the Canarian archipelago. We used population censuses to develop a stage-based demographic model of the species to perform both deterministic and stochastic projections. We also carried out a preliminary essay on the effect of grazing on the population growth. To determine the longevity of individuals we took advantage of dendrochronological techniques as dead collected individuals showed recognizable tree rings. The species showed a clear, but gradual declining trend in its two populations, principally linked to the decrease of yearly rainfall. Survival rates of seedlings were low, and high elasticity values were allocated to the bigger, reproductive individuals, which showed the highest survival rates. The dendrochronological study estimated at 32 years the age of the oldest individual, and in 18 years the average of the 10 dead specimens collected. These results pointed to a lower longevity of individuals with respect to the values inferred by demographic models. The findings of this study suggest that in addition to the preservation of reproductive individuals and their habitat, other measures must be carried out. In particular, the role of grazing needs to be clarified, as seedling survival and recruitment are highly variable. On the other hand, the effects that climate change can play on annual rainfall should be explored along assisted migration measures.

INTRODUCTION

The intrinsic characteristics of volcanic archipelagos make insular biota more vulnerable to extinction due to isolation and limited potential areas, resulting in large amount of endangered species worsening their status at an alarming rate due to several drivers (Millennium Ecosystem Assessment, 2005; Schmid *et al*., 2011; Courchamp *et al*., 2014; Harter *et al*., 2015). Conservation biology have thus put a special focus on islands, given the high concentration of species diversity in such small territories (Kier *et al*., 2009). One paradigmatic case is the Canarian archipelago, which proximity to the African continent has allowed its early colonization by aborigine populations and subsequently by European settlers in the 15th century leading to long disturbance of natural habitats (De Nascimento *et al*., 2009; Fernández-Palacios *et al*., 2011). As part of the Macaronesian biogeographical region, the Canary Islands hosts a large plant diversity with approximatively 50% of endemic taxa (Reyes-Betancort *et al*., 2008) and is considered a significant portion of the Mediterranean biodiversity hotspot (Médail & Quézel, 1999). Threatening factors lurking plant diversity in the Canary Islands encompass a severe habitat fragmentation combined with limited habitats, grazing by introduced herbivores and competition with invasive plant species (Bañares-Baudet *et al*., 2004; Gangoso *et al*., 2006; Caujapé-Castells *et al*., 2010). Thus, over 25% of plant species fall within any of IUCN categories of risk (Moreno-Saiz *et al*., 2015). For the archipelago, the big picture of the conservation studies for individual taxa are rather asymmetric. Conservation genetics have received a strong attention (Caujapé-Castells *et al*., 2010) along with reproductive biology or autoecology (e.g. Sosa *et al*., 2014; Anderson *et al*., 2015). Conversely, although the ensemble of the flora has been assessed under the IUCN criteria (VVAA, 2000; Bañares-Baudet *et al*., 2004; Moreno-Saiz, 2008), published works focusing on demographic studies on viability of the Canarian flora account to date for only six species (Marrero-Gómez *et al*., 2005, 2007; Iriondo *et al*., 2009).

Conservation biology has emphasized that natural history traits, species interactions and the role of species for ecosystem functioning must be addressed beyond the simple conservation of species (Kaiser-Bunbury *et al*., 2010; Devoto *et al*., 2012). Insularity is a driver for less diverse and disharmonic communities (Whittaker & Fernández-Palacios, 2007). This is reflected in the properties of ecological mutualistic networks of the islands, where functional groups may be entirely lost after only one extinction and stability against extinction cascade or community disassembly may be risk factors (Nogales *et al*., 2015; Traveset *et al*., 2015a). Key species for ecosystem processes merit therefore a strong focus. Conservation on islands has frequently overlooked ecological interactions and particularly to plant-animal mutualisms (Kaiser-Bunbury *et al*., 2010). The Canary Islands are not only important as a biodiversity hotspot, but also as a refuge area due to climate stability which has allowed the persistence of relict species and thus they may constitute an important reservoir of phylogenetic diversity as well (Vargas, 2007).

In this paper we focus on one of these examples among the endangered flora of the Canary Islands: *Navaea phoenicea* (Vent.) Webb & Berthel. (Malvaceae) is a rare mallow exclusive from reduced areas in Tenerife Island, where acts as somewhat like a keystone species. This shrub is also a relict species with no direct phylogenetic relationships with the close Malveae tribe (Escobar García *et al*., 2009). In turn, it would be one of the main features of an interesting natural history encompassing a number of singular adaptations, in particular with respect to animal-plant interactions. The species shows a bird-pollination floral syndrome in response to selective pressures and takes part of the Macaronesian Bird-flower Element (Olesen, 1985; Valido *et al*., 2004). This syndrome probably originated by African sunbirds, which has persisted until present, with the generalist Canarian chiffchaff *Phylloscopus canariensis* in the role of the main pollinator. Many dimensions of its natural history are therefore significant either from the conservation perspective or from the evolutionary and ecological disciplines. From the pollinator decline perspective (Cox *et al*., 2013; Tylianakis, 2013), it is fundamental to conserve such rare pollination syndromes, especially given the extinction cascades than can be triggered (Mazykin *et al*., 2011). This bird-pollination syndrome, which appears to be a singular feature of the Macaronesian region may be specially important given the low pollinator redundancy of functions of insular pollination networks (Traveset *et al*., 2015b). Along with phylogenetic distinctiveness, there are other singular traits in this species deserving attention, like insular woodiness which is present in the species. It is indeed a tree or a large sized shrub, for which lignification appears to be an island phenomena (Carlquist, 1974; Lens *et al*., 2013). Although insular woodiness has received

attention from the evolutionary point of view to explain the high frequency of lignification (Lens *et al.*, 2013), growth rings have been overlooked as a prospective tool to complement population viability analysis with few studies for the archipelago (Jonsson *et al.*, 2002). The fundamental habitat is found the transitional bands of thermophilous shrubland between dry coastal desert and humid laurel forests. The flowering period starts in September and ends sparely by January, with few flowers persisting beyond. Seed dispersal is limited mostly to barochory with germination rates remaining low without manual scarification. Lizards can act as occasional seed disperser which facilitate both spatial dispersion of seeds and higher effective germination. The assemble of natural history traits and geographical distribution makes this species a key element of the thermophilous community inhabiting

Table 1. Numbers total mature individuals censed by binoculars in each population and number of individuals (N) within plots in 2006.

Censed individuals			
Sampling year	**Anaga**	**Teno**	**Total**
2006	395	445	840
2015	370	418	788
Individuals within plots in 2006			
Anaga		**Teno**	
Subpopulation	**N**	**Subpopulation**	**N**
Batanes	20	Cecilia	36
Carboneras	16	Cochinos	12
Chamorga	9	Cuevas Negras	11
Chinamada	39	Ravelo	15
Isogue	16	Reparo	12
Taborno	35	Lomo Respingo	22
Taganana	11	Teno	76
Tope Carnero	17		
Total	**163**		**184**

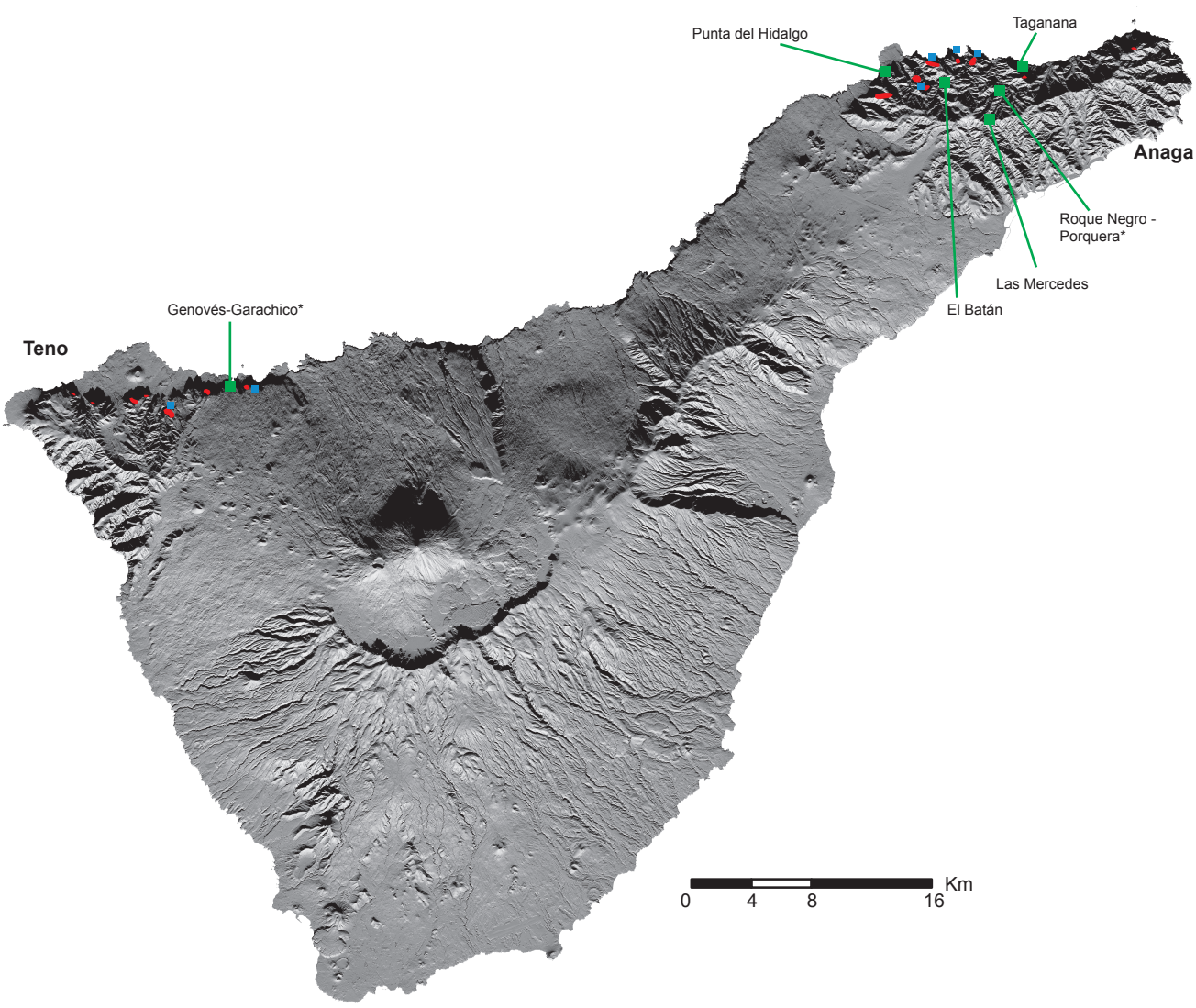


Figure 1. Location of Tenerife island, sampling plots (in red) and situation of meteorological stations employed. Blue points denote the locations of the subpopulations; green squares points denote meteorological stations used. Asterisks denote the stations used as individual climate proxies for λ and ring growth comparisons; blue points were samples for dendrochronology measures were taken.

northern ravines.

Navaea phoenicea has been recognized as a threatened taxon along conservation assessments, fluctuating between Vulnerable and Endangered categories several times (Gómez-Campo, 1996; VVAA, 2000; Rodríguez Núñez *et al.*, 2004; Moreno-Saiz, 2008). Under a legal perspective it is however only considered as "Species of special interest for the Canarian ecosystems" under the scientifically contested (Fernández-Palacios & Nascimento, 2011) regulation established by the Canarian Government (Gobierno de Canarias, 2010). Different evaluations conducted for this plant have identified as main endangerment factors the inability to disperse seeds (Gómez-Campo, 1996), and the effects of grazing, competition with exotic species and habitat loss (Rodríguez Núñez *et al.*, 2004). Indeed, the habitat is restricted to northern slopes of the two old volcanic cores of Tenerife, Anaga (East) and Teno (West) mountain massifs, resulting in only two fragmented populations composed by 8 and 7 subpopulations respectively (figure 1). Census carried out for the Red Book (Rodríguez Núñez *et al.*, 2004) have determined 1400 mature individuals. Subsequent works that performed on the natural history of the species have confirmed the lack of dispersal and germination capacity, but also a limited reproductive success due to the absence of efficient pollinators and limited available habitats. On the other hand, genetic analysis have discarded a low genetic diversity and genetic drift due to the ancestral condition combined with the disjunct distribution and low effective numbers (chapter 2). Despite what has been worked with the species, there are still significant gaps in the knowledge on its conservation status. Vital and demographic parameters such as longevity of the individuals, viability of the populations or the concrete effect of introduced herbivores are studies which have not been accomplished so far. In this paper the aim is to shed light to these questions. For this purpose, a population survey along 10 years was conducted together with a dendrochronological assessment of tree rings to complement age and size within population viability analysis to determine the conservation status of the plant. The specific objectives to test for this species were: 1) to determine population size and population structure; 2) characterize demographic dynamics, trends and variability, and which relationships can be inferred between climate and population growth; 3) incorporate the effect of stochastic variability into the models to determine the fate of populations in different scenarios, and 4) obtain a dendrochronological assessment of the age and ring growth of the individuals and test the relationship between growth and climate.

MATERIALS AND METHODS

Demographic survey

All known subpopulations and nearby areas were prospected and individuals censed. The difficulty to access to the ravines obliged to assemble the total census with binoculars in a large number of occasions. The accessible

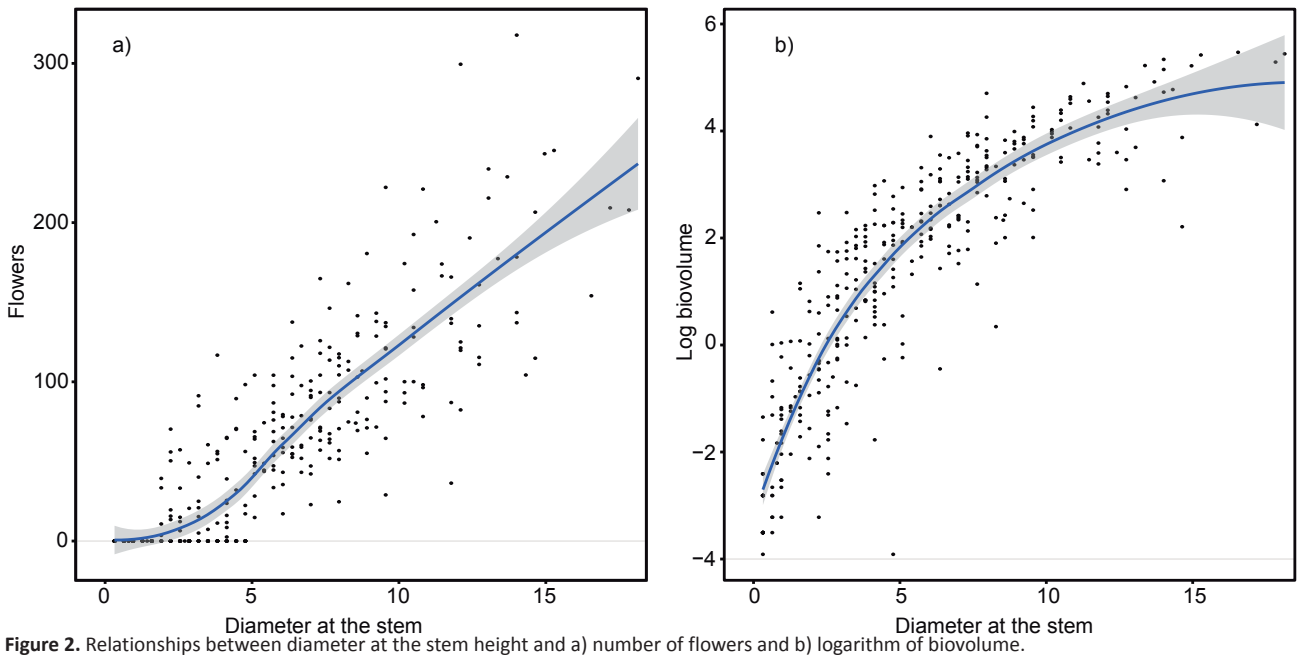


Figure 2. Relationships between diameter at the stem height and a) number of flowers and b) logarithm of biovolume.

Table 2. Results of ANOVA testing effects of locality and year on a) seedling survival and b)recruitment rate.

a) Seedling survival (n=115)			
	df	**F**	**P-value**
Locality	12	3.337	0.00046
Year	9	5.744	2.567
b) Recruitment (n=115)			
	df	**F**	**P-value**
Locality	13	3.292*10-8	2.017*10-7
Year	9	5.097*10-9	3.806*10-16

patches of individuals were subsequently delimited as survey plots for annual demographic monitoring. All individuals within plots were tagged, georeferenced and measured for several biometrical parameters: diameter at the base of the trunk along with height and crown diameter to estimate cylindrical biovolume, as the product of both measures. Flower production and number of branches were also measured. We estimated the correlation between biometrical parameters to determine if diameter at the basis could be accounted as size estimator.

In every annual sampling, plots were visited in search of new seedlings, and biometrical parameters were measured again for every recorded individual. Surveys were performed at the end of the flowering season in late February. Reproductive data coupled with biometry was used to establish the threshold between life stages (see results): 1) seedlings (S) were considered as the first year individuals; 2) juvenile vegetative (J) individuals; 3) juvenile reproductive (JR) as flowering individuals of stem diameter ‹5.8 cm; and 4) mature reproductive (MR), flowering individuals with stem diameter ≥5.8 cm. These classes were used to estimate the transition matrices for demographic modeling along the period of the study. To account for significant differences in seedling survival and incorporation recruitment of new seedlings between localities and years, generalized linear models (GLM) were employed. For recruitment, we assumed that reproductive capacity was directly proportional to tree size. We therefore used the sum of basal diameters as a covariate in the model following Pierson & Turner (1998). With respect to seedling survival, we fitted a GLM with localities and years as predictors. The fate of seedlings (death or survival the next year) was used as dependent variable following a binomial distribution. Finally, georeferenced subpopulations were used to assess IUCN endangerment criteria B1 and B2 with GeoCAT tool (Bachman *et al.*, 2011).

Deterministic matrix modelling and climate variables

Projection matrices were built using popbio package (Milligan & Stubben, 2007) implemented in R software (R Core Team 2013) to calculate transition probabilities between stablished stages. To account for fecundity estimates we took the following approach (Caswell, 2001):. first, the seed production of each individual was calculated and for that purpose the number of flowers was counted weekly in the sampling periods from to 2007-2009. The total of number flowers was used as response variable in a linear regression against diameter of the trunk at the base. The model was used to estimate the total number of flowers of each individual for subsequent sampling years, where the visits to plots occurred only at the end of the flowering period. The total number of flowers of each individual was multiplied by the mean of ovules per flower (21, chapter 3) and the average fructification rate (0.18, chapter 4). Second, the average number of seedlings emerged next year was divided by the average total number of seeds produced every year, as an estimate of the rate of seedling production per seed. Then, the number of seeds produced annually by each individual was multiplied by the mean rate of seedling production, as the individual estimate of fecundity. Lefkovitch transition probability matrices were

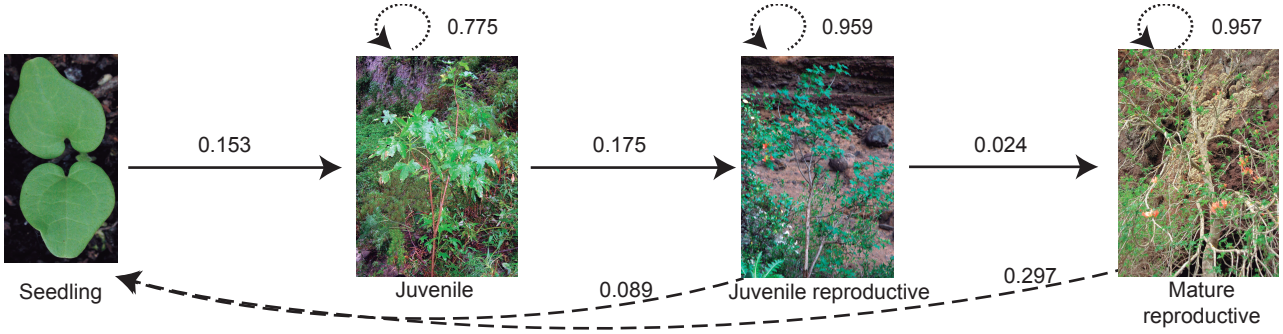


Figure 3. Life cycle of *Navaea phoenicea*. Dashed lines represent fecundity; dotted lines represent survival, and solid lines represent transitions to the next stage.

calculated and subsequently yearly lambda (λ) values of finite growth and bootstrap λ values along with elasticity matrices. Analyses were conducted for the ensemble of individuals and for the two populations Teno and Anaga separately to explore differences in growth rates between them.

In order to test the correlation between growth and climate parameters, datasets of available weather stations from National Meteorological Agency (AEMET) were collected and filtered by location and period of weather records. Only stations within the northern slopes of Teno and Anaga massifs were retrieved for the study. Then, weather stations were restricted to those falling within the altitude of localities of *Navaea phoenicea*. Only one station met the conditions in Teno massif, whereas 5 stations suited the criteria in Anaga (figure 1). Here, the following approach was taken to explore which kind of procedure could be more informative about the environmental influence on population growth. On the one hand, only one station was kept as proxy of the general weather registers for the whole Anaga massif (Carboneras, figure 1). On the other, data from the five stations were used to interpolate a raster map for each monthly variable for every year at 500 m resolution. Calculations were conducted using geoR package (Ribeiro & Diggle, 2015) in R. The values obtained for the centroid cell of all subpopulations in Anaga were then used as climate proxy. Monthly, quarterly, average and total precipitation values and maximum, minimum and average temperature in Teno and Anaga separately, were used to test the significance of the correlation between λ and climate values.

Stochastic simulation of population dynamics

Stochastic simulations were conducted again with *popbio* package which implement equations for stochastic models from Morris and Doak (2002). In order to account for potential environmental variability affecting growth rates, three scenarios were developed to assign probabilities to years: one stable scenario where all yearly matrices were weighted equally; a negative scenario where matrices of years with lambda<0 were given twice the weight of those of years with λ>0; and a third, positive scenario where conversely, years with positive growth were given double weight in the stochastic simulations. Models were run with 50.000 iterations. Quasi extinction probabilities were calculated on the basis of an extinction threshold of N=100 individuals and a temporal horizons of 100 years.

Grazing exclusion experiments

Exclusion experimental plots were set up in the two localities were topography allowed to conduct the experiment, and hosts a large number of individuals, Taborno in Anaga and Teno Alto in Teno massif. Two contiguous plots of approximately 0.25 ha were established in each locality. One was protected against

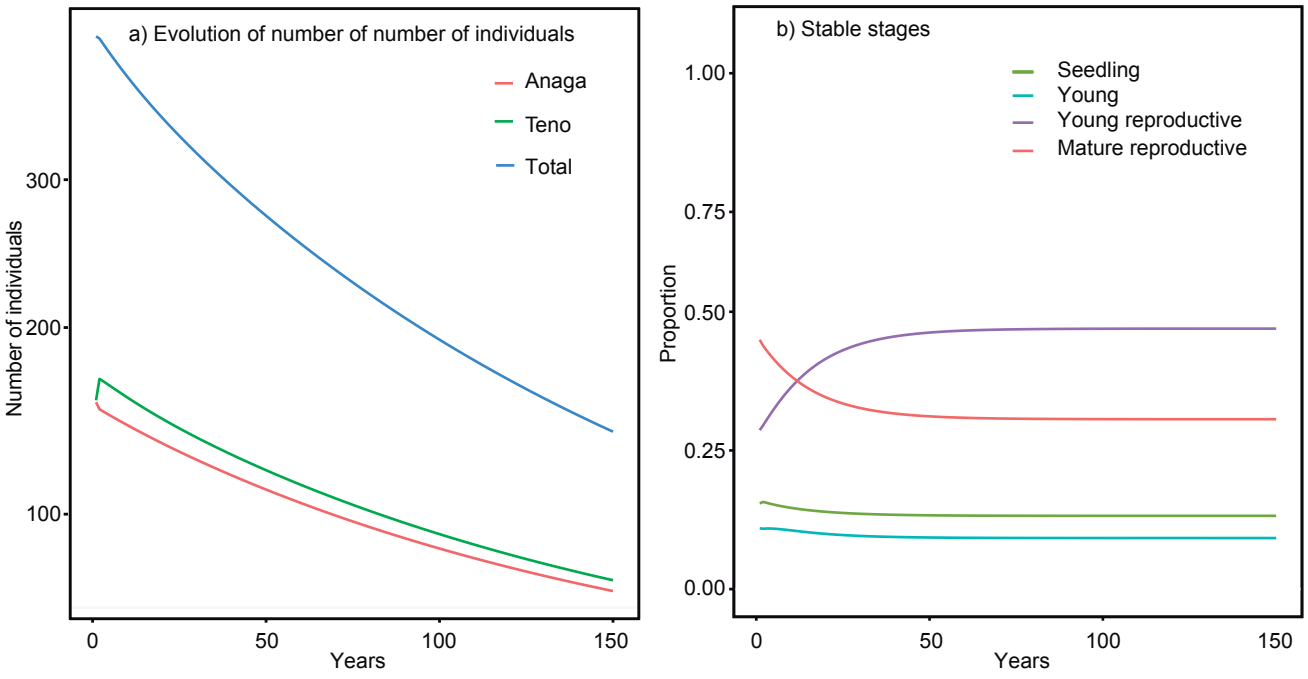


Figure 4. a) Deterministic trajectory projection of N. phoenicea monitored populations from the number of individuals in 2015 for a period of 150 years. b) Deterministic projection for the proportions of each life stage from the 2015 population vector for a 150 year period.

grazing with chicken mesh while the other was designed as control. Seedling survival in both plots was monitored for two consecutive years. Exclusion plots were not taken into account into demographical models. For each plot we counted the number of surviving seedlings. Differences in number of survivors were tested with a linear model following a binomial distribution.

Dendrochronological analyses

Given the current conservation status of the species, only dead individuals were used in the dendrochronological assessment. Samples were obtained from the beginning of the survey to 2009. Dead trunks were sliced at the basis of the stem. Slices were then manually sanded and processed to make evident the tree rings in the stem to later proceed to count the growth rings and measure them. The rings in the treated slices were subsequently counted and measured with a Velmex sliding-stage micrometer. For each sample we measured rings along three radii, and average ring width was calculated. The individuals for which the date of death was known were used to establish a master chronology. Those individuals that were found dead at the beginning of the study and which death year was therefore unknown, were matched to the highest correlation coefficient with the master chronology. All calculations were performed with dplR package in R (Bunn, 2008).

AEMET meteorological stations were used again to explore the relationships between climate and stem growth. The set of meteorological records was filtered to select stations accounting for data for the life time period of the sampled specimens. Finally, two stations were chosen as climate proxies (figure 1) for Anaga and Teno specimens. The significance of correlations with climate was tested to explore the effects of climatic variables on growth rate. As for finite growth rates, the significance of correlation coefficients were computed for monthly minimum, maximum and mean temperature, and monthly and total annual rainfall.

RESULTS

Demographical survey and population structure

Initial survey detected 840 individuals, about half of the 1400 counted in previous census (table 1). However, the survey in inaccessible plots with binoculars could only account for flowering individuals, but not seedlings

Table 3. Average projection and elasticity matrices, average and minimum-maximum bootstrapped λ values for the complete period for Teno and Anaga and the population ensemble. S=seedling, Y= juvenile non reproductive, JR= juvenile reproductive, MR= mature reproductive. N= mean time spent in each stage.

	Projection matrix				Elasticity				λ	Boot. λ
Teno										
	S	**Y**	**YR**	**MR**	**S**	**Y**	**JR**	**MR**		
S	0.000	0.000	0.092	0.284	0.000	0.000	0.010	0.010		
Y	0.185	0.7599	0.000	0.000	0.020	0.065	0.000	0.000	0.992	0.985 1.000
JR	0.000	0.192	0.963	0.000	0.000	0.020	0.653	0.000		
MR	0.000	0.000	0.016	0.946	0.000	0.000	0.012	0.210		
N	1	4.71	21	30						
Anaga										
S	0.000	0.000	0.083	0.297	0.000	0.000	0.002	0.013		
Y	0.110	0.787	0.000	0.000	0.016	0.062	0.000	0.000	0.994	0.937 1.000
JR	0.000	0.158	0.951	0.001	0.000	0.016	0.386	0.000		
MR	0.000	0.000	0.0375	0.966	0.000	0.000	0.014	0.486		
N	1	4.18	27.30	18.63						
Total										
S	0.000	0.000	0.089	0.291	0.000	0.000	0.006	0.012		
Y	0.153	0.775	0.000	0.000	0.010	0.067	0.000	0.000		
JR	0.000	0.177	0.959	0.000	0.000	0.002	0.523	0.000	0.994	0.962 1
MR	0.000	0.000	0.024	0.957	0.000	0.000	0.013	0.339		
N	1	4.44	24.65	23.54						

or juvenile individuals, which could not be easily detected. Within the plots stablished, starting number of individuals was 349, 163 and 184. Annual growth inferred from the differences between diameters measured in consecutive years was on average 1.92 and standard deviation 1.01. Number of flowers and logarithm of the biovolume were significantly correlated with basal stem diameter ($F=910.5$, $p=2.2e\text{-}16$, $R^2=0.857$; $F=558$, $p=2.2e\text{-}16$, $R^2=0.780$, $n=349$, figure 2a and b respectively). Diameter was therefore considered a valid measure to establish size classes. Proportion of seedling survival from one year to the next ranged from 0 to 100% with a mean of 0.15. Survival rate significantly varied between years and subpopulations (table 2a). For recruitment, the minimum ratio of seedlings emerged/ seeds produced ranged from 0 to $0.587*10^{-3}$. Linear models also detected significant differences for both predictors (table 2b).

The analysis with GeoCAT tool identified an extent of occurrence (EOO) of 185.443 km^2 and an area of occupancy (AOO) of 48.000 km^2. These numbers delivered the EN IUCN category for criteria B1 and B2 respectively.

Deterministic matrix modelling, fecundity and survival rates

Average λ values for the whole population ranged from 0.9821 (2012) to 1.022 (2010) (table 3, supplementary table 1). In Teno and Anaga, average λ was 0.992 and 0.994

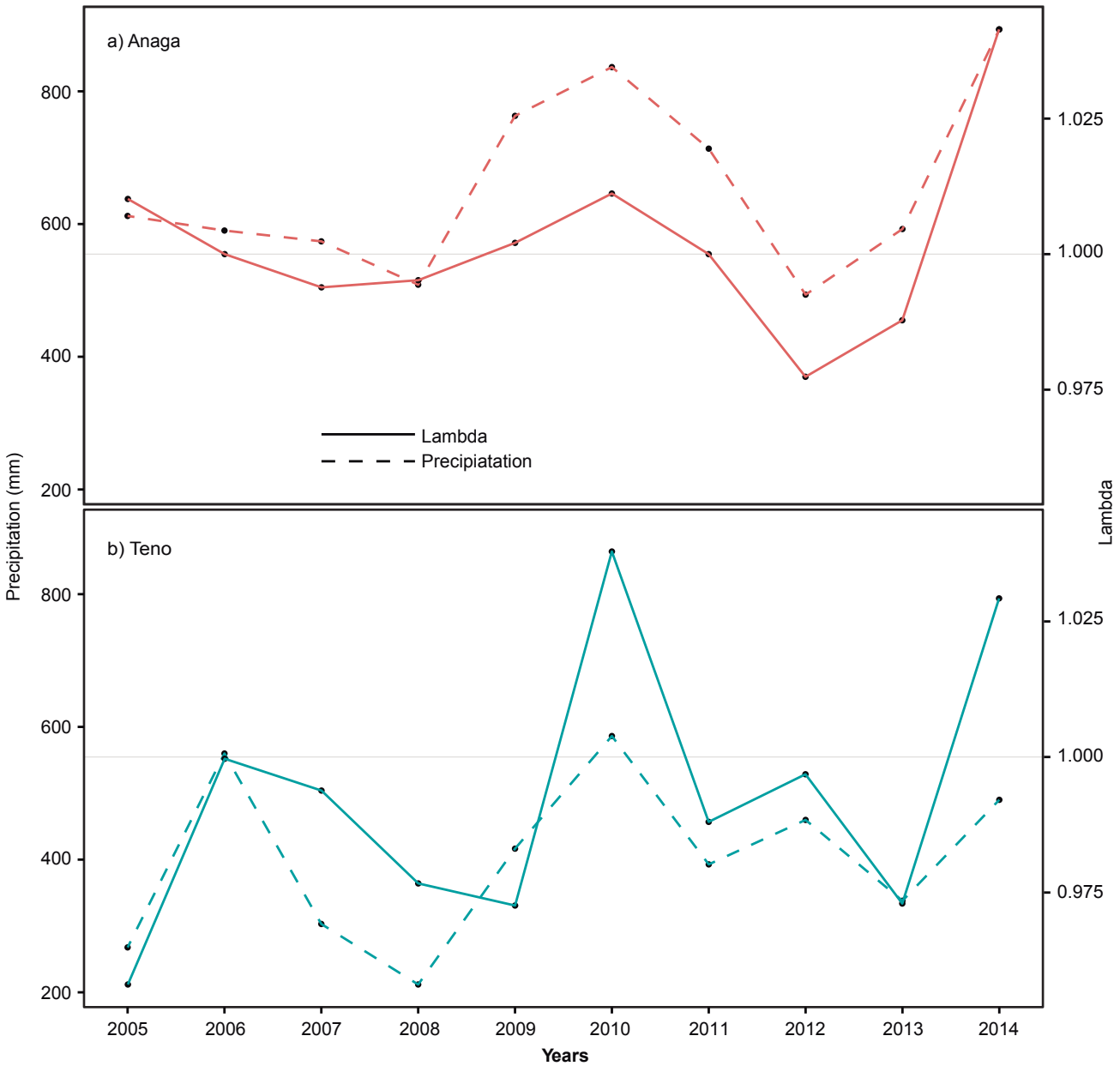


Figure 5. Yearly lambda growth rates for Anaga and Teno populations (black line) and yearly total precipitation values for meteorological stations (dashed lines).

respectively, and for the total population the value was 0.994. Bootstrapped values ranged overall from 0.985 to 1; 0.937 to 1 in Teno and 0.962 to 1 in Anaga. Altogether, 3 transitions showed λ>1 in Anaga (2009-2010, 2010-2011 and 2014-2015), 2 in Teno (2009-2010, 2013-2014), and 1 for the ensemble (2009-2010). Figure 3a represents the values of fecundity, transitions rate to the next stage and survival rates within a given stage. We did not detect any reversion to former stage or size degrowth. Survival rates were higher in the reproductive stages. The transition from seedling to juvenile, i.e., seedling survival, showed lower values. Fecundity was fairly higher in mature reproductive individuals than in juvenile reproductive individuals (table 3). High values for elasticity were found for survival of reproductive individuals and low values for the fecundity elements of the matrix. As a result of an average □<1, the deterministic matrix model projected a population decrease (figure 3b). The current stage structure differed from the stable structure defined by the population matrix essentially only between juvenile and mature reproductive individuals as the distributions of their proportions got inverted. Distributions of seedlings and juvenile individuals decreased slightly (figure 3c). Time spent in each stage by an individual, estimated from the projection matrix, indicated that *Navaea phoenicea* is a long-lived species: average times were 1 year in the seedling stage, 4 years in the juvenile stage, 24 as juvenile reproductive and 25.54 as mature reproductive. This was in sum 55 years. The times estimated for the mature reproductive stage were largely less in Anaga (18) than in Teno (30).

No monthly variables of temperature or precipitation presented any significant correlation with yearly λ values. Conversely, total annual precipitation showed a strong relationship with λ (figure 4). In Teno, Pearson R^2 was 0.764 (t=3.35, df=8, p=0.0109). In Anaga, where two distinct approaches were conducted, results differed. In contrast with Teno, there was a weak correlation for a single station (R^2 = 0.401, t=1.24, df=8, p=0.249). For the centroid of the interpolated values however, correlation was strong again (R^2 = 0.818, t=4.026, df=8, p=0.004). Elasticities were high for the survival of juvenile reproductive and mature reproductive individuals. More frequently, annual elasticity matrices assigned higher values to the survival of juvenile reproductive individuals than mature reproductive individuals. However these values varied widely; in several periods elasticity of survival of mature individuals reached 1, while in others, conversely juvenile reproductive individuals achieved 1 as well. Transition between stages or fecundity showed very low values.

Stochastic projections

Table 4 summarizes stochastic projections under the defined negative, stable and positive scenarios for a temporal horizon of 100 years. Growth rates calculated for stochastic projections showed that at any of the three defined scenarios both in total and for the two populations, its value was always <1, although in the positive scenario, λ was 0.999. Quasi-extinction probabilities varied between scenarios (figure 5a). Under the negative scenario the probability was over 0.938 in all cases; under the stable scenario probability in Teno and Anaga separately was also 1, but not for the ensemble (0.154); finally, the positive scenario gave null probability of quasi-extinction to the population ensemble, low for Teno (0.184) and intermediate for Anaga (0.570). For shorter time horizons, the probabilities for the two populations separately only started to increase after 25 years, not before.

Projected population numbers at the end of the simulations were very variable (figure 5b). For both Anaga

Table 4. Results of stochastic projections for a 150 years horizon. Lambda approximate values and projected number of effectives are displayed in bold between the 95% confidence interval. For quasi-extinction probabilities, only the mean value is provided as variation was almost 0 in the three scenarios.

		Negative scenario	Stable scenario	Positive scenario
Teno	Lambda	0.989	0.993	0.999
	Projected population numbers	25±8	57±20	163±59
	quasi- extinction probability	0.99	0.973	0.184
Anaga	Lambda	0.990	0.993	0.994
	Projected population numbers in 150 years	45±8	63±11	101±18
	quasi- extinction probability	1.000	0.997	0.570
Total	Lambda	0.990	0.994	0.999
	Projected population numbers (mean ± sd)	121±22	181±37	315±67
	quasi- extinction probability	0.938	0.154	0.000

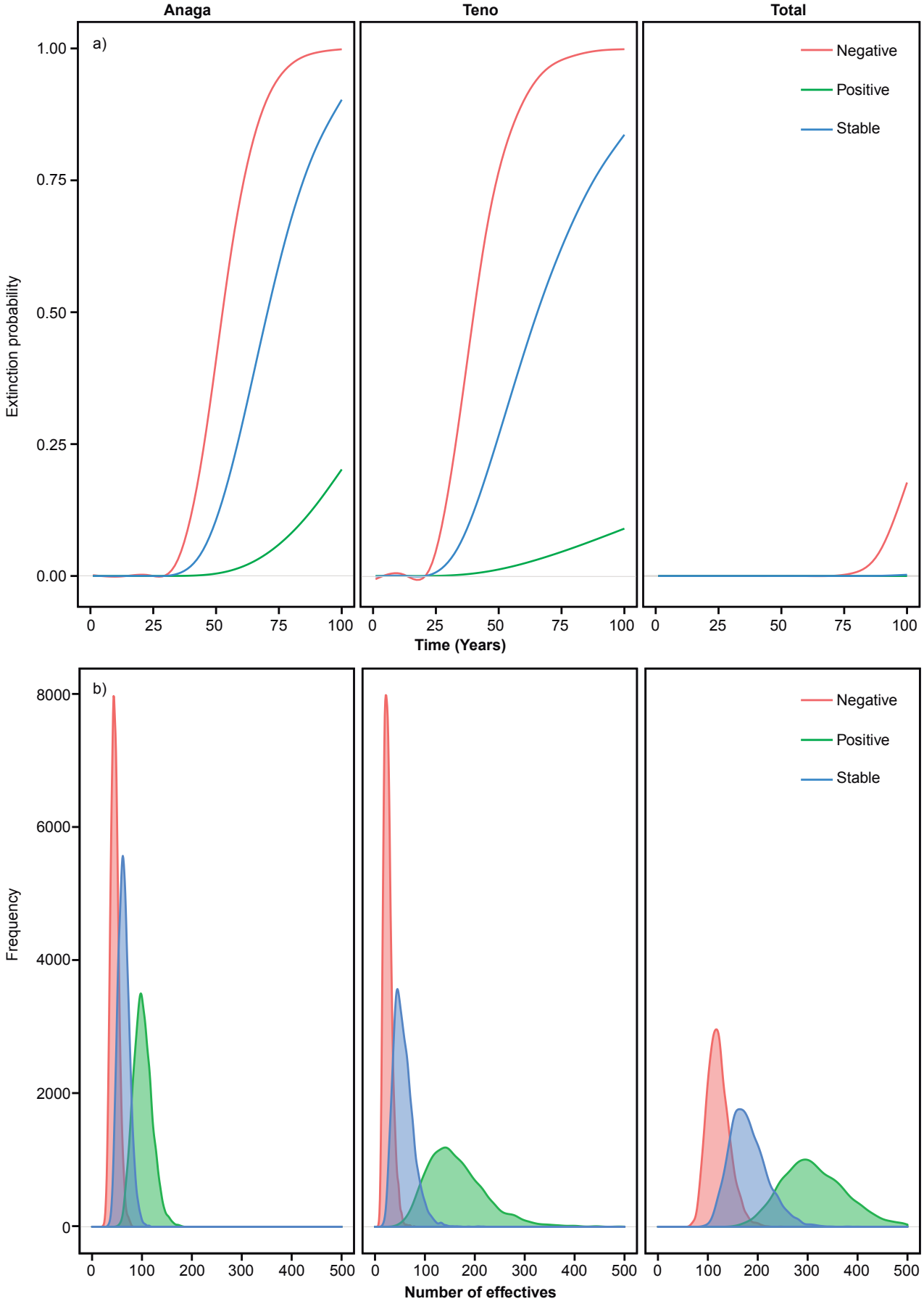


Figure 6. a) Quasi-extinction probabilities for 100 years period performed for Anaga and Teno populations separately and the population ensemble. The probabilities predicted by the stochastic model for the three scenarios (negative, stable and positive) are plotted against time. b) Density of frequency of population numbers at year 100 predicted by the model for the three scenarios.

and Teno separately, only in the positive scenario the most probable number of effectives was over 100. Accounting for the total population, the most probable population numbers were 121 in the negative scenario, 181 in the stable, and 315 in the positive scenario.

Grazing exclusion experiments

The exclusion treatment led to a significant higher seedling survival (df=1, F= 27.667, p=2.37*10^{-7}). In Taborno subpopulation 13% of seedlings survived while 14% did in Teno. In contrast, only 1% of seedlings survived without exclusion in Taborno and 0% in Teno without exclusion in exchange. There were no significant differences between plots (P= 0.989) nor interaction between factors (P=0.667).

Dendrochronological analyses

Ten dead samples were obtained for the sampling period 2005-2008, eight in Anaga and two in Teno. Along annual surveys, only eight dead individuals could be recovered, and two were found dead at the beginning of the monitoring period. The existence of rings was confirmed (figure 7), although the chronological series were generally very short. The oldest sample was REP01 with 32 years and the youngest was TAB04 with 11 (figure 6b). The mean age of sampled individuals inferred from rings was 18.09, fairly below the times inferred from the projection matrix. On average, annual growth rate was 2.011 mm and standard deviation 1.17, slightly higher than growth rate measuring at the stem base. Pearson correlation of the individual growth rates was 0.61.

No significant correlations were found between average growth per population and any of the temporal horizon considered for precipitation or temperature. Figure 6c shows the relationship between ring growth and total precipitation for the period 1978-2010. In Anaga, the correlation between growth and precipitation appeared to be correlated only in the period 1999-2001, and in Teno between 2004 and 2006. However at some parts of the chronological series, a time lag in the covariation between rainfall and growth could be observed, suggesting that a stronger relationship between climate and growth might happen, but weakly coupled across years.

DISCUSSION

This study provided the first approach for a long-term population survey in the thermophilous Canarian shrubland and contributes to the understanding of risk factors for the endangered insular taxa. These results, which account for a representative sample of the whole population, offer insights both on the size-structure of the population and which constraints, climate or grazing, are driving current population trends, which show a slow decline. The analysis has been carried out for a key species for the ravine habitats and constitute the first viability study for an endangered species of the Canarian thermophilous forest, which moreover exhibits the phenomena of insular woodiness.

Figure 7. a) Section of the stem with visible rings. b) detail of wood radii.

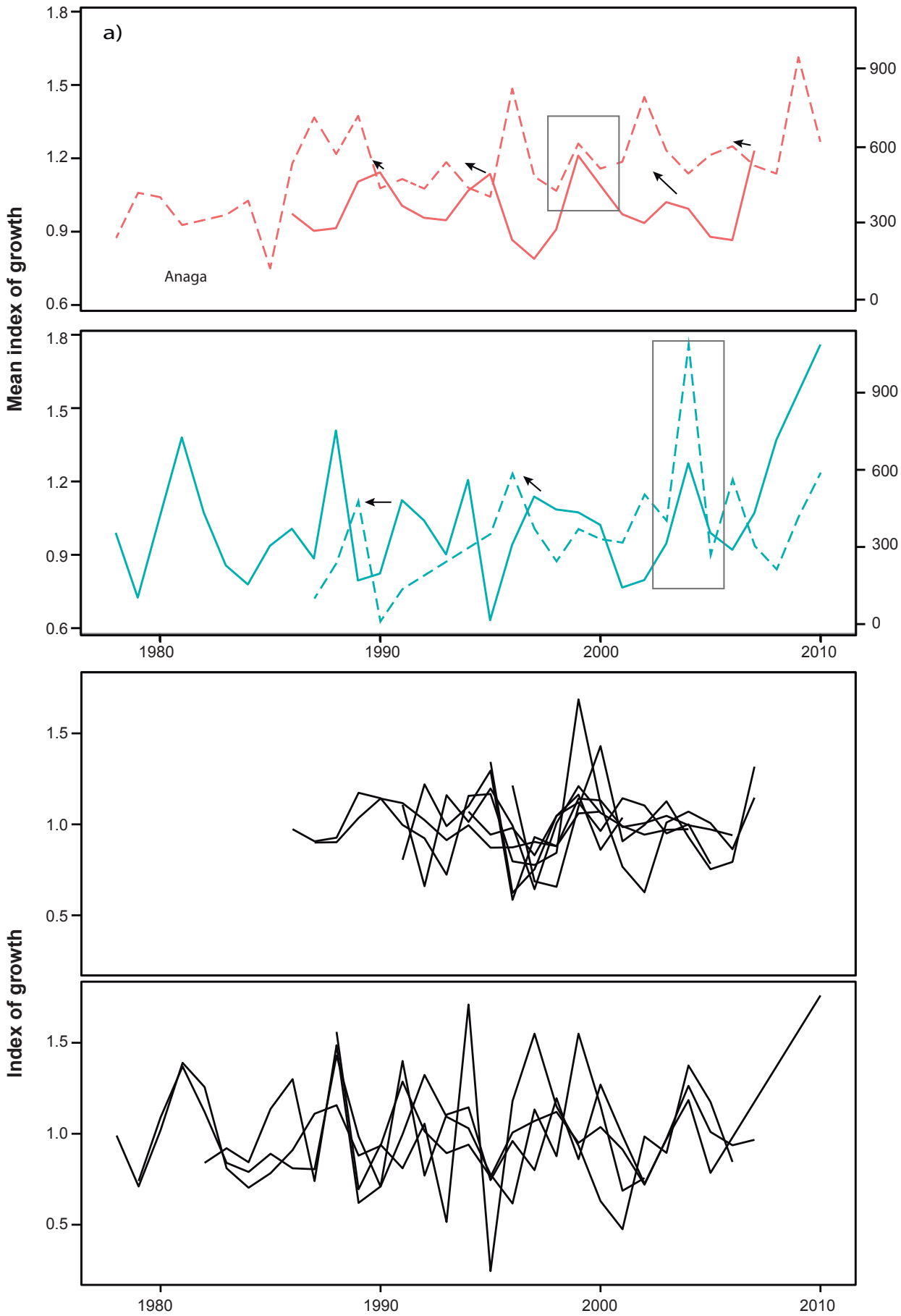


Figure 8. a) Index of growth per individual through time. Dashed lines represent total precipitation. Grey frames show years with high correlation between growth and precipitation. Arrows indicate possible time offsets in the potential relationship between precipitation and growth, if it actually happens. b) Growth rate index for all the individuals sampled.

Size and population structure

There was a large discrepancy between the census accounted by this study and a previous one made on the occasion of the Red Book (Rodríguez Núñez *et al.*, 2004). Our figure is merely only half of the individuals previously estimated. A strong decline in population numbers is not likely to have happened, given the slow decline trend found here. Instead, discrepancies are more likely to do with the use of different estimation methods and the existence of look-alike species at the same locations. For instance, conspicuous flowers can be confused in the distance with feral ornamental red geraniums, and highly aggregated individuals can be wrongly counted if not prospected closely.

Even with already mentioned accessibility problems to some localities, the sample size that could be surveyed within plots achieved to represent a fraction of approximately half of the total population census. Population structure based on size in *Navaea phoenicea* was dominated fundamentally by mature individuals. Because in most years annual surveys were performed once, it is very likely that annual data only accounts for a fraction of total seedlings in the population, although the sampling moment was chosen to maximize number of seedlings emerged from the seed bank. Accounting for the total number of seedlings emerged throughout the year may confer to the species population the classical J-shaped distribution curve of frequency of each stage. From the perspective of life stages, collected data would classify as "senile" populations (Oostermeijer *et al.*, 1994). Juvenile individuals were present in very low numbers and were scarcer than seedlings. The distribution of selected size classes gives a first indication of population dynamics. It points indeed to a restriction in the recruitment, due to a lack of seedling survival, which may preclude regeneration of populations.

Projected population trends and drivers. Reasons for concern?

As expected, the variability of finite growth rate across years and populations was always close to 1, indicating the stability of population. This stability is due to the longevity and relative slow growth of individuals, which drive little variations in population numbers and transitions to the next stage between years. The average trend in both populations projected however a slight decline of the population. Even though modelling in this study account only for the individuals surveyed at plots and not for the whole census, predictions under the deterministic modelling show a number of less than 100 individuals within the next century. This projection allow to infer a reduction of 30% of the population in the next 50 years. Under the IUCN criteria A, which accounts for drastic reduction of mature individuals, the species would not be considered as threatened.

The COMPADRE database (Salguero-Gómez *et al.*, 2015) allows to retrieve population matrices for comparisons, but unfortunately other pertinent case studies are scarce. Within the Malvaceae family, few species have been prospected and none of them shares same type of arborescent habit: Viability of *Abutilon theophrasti* has been assessed as a weed in North America (Westerman *et al.*, 2005). The forb *Sphaeralcea coccinea* in its native range in North America shows an average λ=1 and a decline in the population growth with climate change (Dalgleish *et al.*, 2010). Finally, the rare *Kosteletzkya pentacarpos* in eastern Spain achieves a λ=1.03 (Pino *et al.*, 2007). With respect to islands, even less studies can be found, with only one species in Hawaii (DeWalt, 2006). Despite the scarcity, the Canary Islands are then the best studied oceanic archipelago from the viewpoint of population viability researches. Among the known population viability analyses performed for the Canarian plants (Marrero-Gómez *et al.*, 2005, 2007; Iriondo *et al.*, 2009), *Navaea phoenicea* showed by far the lowest fluctuations in annual population growth. Overall, the studied taxa, which are mostly located in the thermophilous shrubland, showed in general a higher declining trend. However, studies accounting for drivers of population trends are only reduced to *Helianthemum juliae*, a small shrub situated in the Cañadas del Teide National Park in Tenerife, at an altitude of 2700 m a.s.l. (Marrero-Gómez *et al.*, 2007). For this species, highest elasticity was also associated to survival rates of mature individuals. Likewise survival of juvenile individuals was associated to rainfall amounts. For the relict tree *Prunus lusitanica*, the optimal climate conditions of Macaronesian laurel forests favor higher sexual regeneration of the populations (Cáceres-Escudero, 2014).

Accounting for stochasticity under different scenarios of population growth, only the positive scenario did not predict the quasi extinction of the species in the "short" term. The probabilities are dependent of two arbitrary inputs, one is the time period for the calculation, and the second one is the departing number of individuals. Here

the models were run accounting only for individuals within survey plots, but which achieve to represent approximately 50% of the total estimated population, thus actual quasi-extinction probability should be lower. In the three scenarios however, average stochastic lambda was always below 1, indicating a likely decrease of the number of effectives regardless the environmental variability. Stochastic models allow assessing IUCN criterion E (›20% of extinction probability in less than 20 years or 5 generations) for probability of extinction within a time period. Given the long lifespan of cohorts estimated by the matrix models, extinction probability is not likely to increase in the next 25 years, but in 50. This corresponds to the time period of about 2 generations according to the matrix calculations; therefore the *Navaea phoenicea* should be included in EN category under this criterion.

The causes of the decline may be twofold. First, demographic analysis showed a strong relationship between precipitation and λ, where years with precipitations ›550 mm drove a positive population growth. Overall, the elasticity analysis showed that reproductive stages of individuals are crucial for the survival of populations as they got significantly higher values than any transition or fecundity values in the average matrices. On the other hand, the congruence between recruitment and rainfall has been found in Tenerife for other species in different climate regimes and habitats (Marrero-Gómez *et al.*, 2007; Fernández-Lugo *et al.*, 2015) This is consistent with the fact that juvenile individuals show a high survival rate, this is, once the seedling period has been overcame. The second possible factor is seedling predation as a direct driver against recruitment. Although grazing is considered a recurrent risk factor for the Canarian flora (Moreno-Saiz *et al.*, 2015), here evidence for this is uncertain, specially confronted with findings for the effect of rainfall. Even if the preliminary experiment showed significant differences in the recruitment between exclusion and non-exclusion plots, the little sample size prevents to draw any conclusions. Moreover, this experiment does not take into account either spatial or annual variability. Because recruitment and seed survival vary significantly between populations and years, there may be several local factors affecting recruitment.

The average high elasticity attributed to reproductive individuals indicates a requirement for the conservation of the species, which can be translated into recommendations of habitat conservation. This offer challenges for decision-makers, as land management in islands can bring particular difficulties (Fernandes *et al.*, 2015). However, under the current trend of populations and probabilities this may not be enough in the long term. Since recruitment appears to rely largely on climate, it is necessary to assess current and future trends of precipitation. Trends from preindustrial times until present show a significant decrease in the precipitation for the Canary Islands when measuring tendencies at the regional scale (Niang *et al.*, 2014) but not significant at the Macaronesian scale (Cropper & Hanna, 2014). Projections of IPCC scenarios (IPCC Working Group I, 2013) predict a significant decrease of rainfall of at least 20% by 2100, but only under the worst emissions scenario. IPCC 5 scenarios have not been downscaled for the Canary Islands but other initiatives provide some insights that account for regional climate change. For instance, the *clima impacto* project (www.climaimpacto.eu) also supports similar scales of precipitation decrease using previous climate models. The complexity of climate in oceanic islands brings however the need to downscale the data to represent microclimate variables that operate at finer scales (Harter *et al.*, 2015). For instance, the effect of trade winds, which are crucial for rainfall regime and water balance, may be very difficult to model for present climate and to downscale future scenarios. It has been predicted a potential downward shift of the cloud forest resulting from the incidence of trade winds (Sperling *et al.*, 2004), which may in turn favor those subpopulations of *Navaea phoenicea* in the lower altitudes. On the other hand, the species distribution shows a certain altitudinal variation, with subpopulations located in the lower edge of the thermophilous shrubland limiting with the coastal desert, and others within the laurel forest. Therefore, the fate of populations may be asymmetric depending on the distribution under scenarios of intense climate change, but can bring an opportunity to carry out assisted migration.

Coupling dendrochronology and population viability analysis

Although evolutionary implications of insular woodiness in *Navaea phoenicea* are beyond the scope of this paper, accounting for tree rings and stem growth allowed to confirm the existence of true woodiness in a so far disregarded species. Rings could be easily visualized and measured. Because of the conservative approach to an endangered species, sample size achieved throughout the period was very low, therefore these results should be discussed with caution.

Although the correlation between chronological series was satisfactory, the relationship between climate variables and tree growth was uncertain. This weak correlation may be due to the inability of meteorological stations to reflect local microclimatic effects linked to topography, which could better explain ring growth patterns. Measures of annual growth taken in the sampling plots were inconsistent with width increments measured from tree rings. Similarly, there was no correlation between age and diameter. Age therefore, and as often it happens in the vast majority of species studied, can be considered a poor predictor of the reproductive ability of individuals, which is in turn rather correlated with size. A second inconsistency found between matrix modelling and this dendrochronological study is the difference in the survival rates found. Even with this low sample size, it should be accounted as a warning given the conservationist scope of this paper. The mean age of dead individuals was 18 years and the oldest individual, which also had the second largest trunk, was 32. On the contrary, matrix modelling gave much higher estimates of survival in each stage. Since conservation strategies rely partially on the assurance of survivorship of largest individuals, this incongruence must be taken into account as a caution measure, if living times extracted by matrix modelling are overestimated.

Concluding remarks for conservation strategies

The findings of this study identify a population decline driven by a lack of recruitment in the driest years, which were frequent in the survey period. The application of IUCN criteria through this study has contributed to bring evidence to maintain *N. phoenicea* under the EN category. The rate of decline, although clear, is modulated by the long lifespan of the species. Even in the absence of such risk of rapid decay, which would better suggest a long term conservation strategy, it is necessary to address the remaining risk factors. Population numbers are low and subject of catastrophic events. These can be natural processes frequent in the oceanic archipelagos, such as landslides or volcanic episodes, but also can be human induced impacts: grazing, together with non-climatic factors affecting seedling survival for instance, still needs to be addressed in an accurate manner; habitat preservation and competition with invasive species needs to be addressed as well, as part of the conservation practices for reproductive individuals. Climate-induced changes in vital rates may be more difficult to address. But besides assisted approach, habitat conservation does not only contribute to the preservation of reproductive individuals, it should be preserving bird pollinators and ensuring reproductive fitness, which may improve with higher visit rates of efficient pollinators. Although time-consuming, population viability analyses prove to be useful for a better understanding of the risk factors that threaten populations. Given the singularity of the island biota and the conservation status, it is therefore necessary to improve the use of these methods.

REFERENCES

Anderson G.J., Bernardello G., & Santos-Guerra A. (2015) Reproductive biology of *Solanum vespertilio* (Solanaceae), a zygomorphic, heterantherous, enantiostylous, and andromonoecious rare Canary Islands endemic. *Plant Systematics and Evolution*, **301**, 1191–1206.

Bachman S., Moat J., Hill A., de la Torre J., & Scott B. (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *ZooKeys*, **150**, 117–126.

Bañares-Baudet Á., Blanca G., Güemes J., Moreno-Saiz J.C., & Ortiz S. (2004) *Atlas y Libro Rojo de la Flora Vascular Amenazada de España*. Dirección General de Conservacón de la Naturaleza, Madrid.

Bunn A.G. (2008) A dendrochronology program library in R (dplR). *Dendrochronologia*, **26**, 115–124.

Cáceres Escudero Y. (2014) *Macroecología de la reproducción en Prunus lusitanica L.: variabilidad geográfica y efectos de la marginalidad en un árbol relicto*. Universidad de Extremadura,

Carlquist S. (1974) Insular woodiness. *Island Biology* pp. 350–428. Columbia University Press, New York.

Caswell H. (2001) *Matrix Population Models: Construction, Analysis and Interpretation*. Sinauer, Sunderland.

Caujapé-Castells J., Tye A., Crawford D.J., Santos-Guerra A., Sakai A., Beaver K., Lobin W., Vincent Florens F.B.B., Moura M., Jardim R., Gómes I., & Kueffer C. (2010) Conservation of oceanic island floras: Present and future global challenges. *Perspectives in Plant Ecology, Evolution and Systematics*, **12**, 107–129.

Courchamp F., Hoffmann B.D., Russell J.C., Leclerc C., & Bellard C. (2014) Climate change, sea-level rise, and conservation: keeping island biodiversity afloat. *Trends in Ecology & Evolution*, **29**, 127–130.

Cox H.M., Taylor M.A., Evans L.J., Mcbrydie H.M., Thurman W.N., Burgett M., Harder L.D., Aizen M.A., Cunningham S.A., Harder L.D., Aizen M.A., Aschero V.,

Chacoff N.P., Kremen C., Tscharntke T., Tylianakis J.M., Kremen C., Hoehn P., Kremen C., Functioning E., Bunker D.E., Hector A., Loreau M., Perrings C., & Naeem S. (2013) Plant-pollinator interactions over 120 Years: Loss of Species, co-occurrence, and function. *Science*, **339**, 1611–1616.

Cropper T.E. & Hanna E. (2014) An analysis of the climate of Macaronesia, 1865-2012. *International Journal of Climatology*, **34**, 604–622.

Dalgleish H.J., Koons D.N., & Adler P.B. (2010) Can life-history traits predict the response of forb populations to changes in climate variability? *Journal of Ecology*, **98**, 209–217.

Devoto M., Bailey S., Craze P., & Memmott J. (2012) Understanding and planning ecological restoration of plant-pollinator networks. *Ecology Letters*, **15**, 319–328.

DeWalt S.J. (2006) Population dynamics and potential for biological control of an exotic invasive shrub in Hawaiian rainforests. *Biological Invasions*, **8**, 1145–1158.

Escobar García P., Schönswetter P., Fuertes Aguilar J., Nieto Feliner G., & Schneeweiss G.M. (2009) Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). *Molecular Phylogenetics and Evolution*, **50**, 226–39.

Fernandes J.P., Guiomar N., & Gil A. (2015) Strategies for conservation planning and management of terrestrial ecosystems in small islands (exemplified for the Macaronesian islands). *Environmental Science & Policy*, **51**, 1–22.

Fernández-Lugo S., de Nascimento L., Méndez J., González-Delgado G., Gomes E.P.C., Otto R., Arévalo J.R., & Fernández-Palacios J.M. (2015) Seedling survival patterns in Macaronesian laurel forest: A long-term study in Tenerife (Canary Islands). *Forestry*, **88**, 121–130.

Fernández-Palacios J.M. & de Nascimento L. (2011) Political erosion dismantles the conservation network existing in the Canary Islands. *Frontiers of Biogeography*, **3**, 99–103.

Fernández-Palacios J.M., De Nascimento L., Otto R., Delgado J.D., García-del-Rey E., Arévalo J.R., & Whittaker R.J. (2011) A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography*, **38**, 226–246.

Gangoso L., Donazar J.A., Scholz S., Palacios C.J., Hiraldo F., & Donázar J. A. (2006) Contradiction in conservation of island ecosystems: Plants, introduced herbivores and avian scavengers in the Canary Islands. *Biodiversity and Conservation*, **15**, 2231–2248.

Gobierno de Canarias (2010) Catálogo Canario de especies protegidas. **167**, 16.

Gómez-Campo C. (1996) *Libro Rojo de especies vegetales amenazadas de las Islas Canarias.* Viceconsejería de Medio Ambiente, Gobierno de Canarias, Santa Cruz de Tenerife.

Harter D.E.V., Irl S.D.H., Seo B., Steinbauer M.J., Gillespie R.G., Triantis K. a., Beierkuhnlein C., & Fernández-Palacios J.M. (2015) Impacts of global climate change on the floras of oceanic islands – Projections, implications and current knowledge. *Perspectives in Plant Ecology, Evolution and Systematics*, **17**, 160–183.

IPCC Working Group I (2013) IPCC Fifth Assessment Report (AR5) - The physical science basis. *IPCC*, .

Iriondo J.M., Albert M.J., Giménez-Benavides L., Domínguez Lozano F., & Escudero A. (2009) *Poblaciones en peligro: Viabilidad demográfica de la Flora Vascular Amenazada de España.* Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino), Madrid.

Jonsson S., Gunnarson B., Criado C., Geology Q., & Laguna L. (2002) Drought is the major limiting factor for tree-ring growth of high-altitude Canary Island pines on Tenerife. *Geografiska Annaler Series A-Physical Geography*, **84A**, 51–71.

Kaiser-Bunbury C.N., Traveset A., & Hansen D.M. (2010) Conservation and restoration of plant-animal mutualisms on oceanic islands. *Perspectives in Plant Ecology, Evolution and Systematics*, **12**, 131–143.

Kier G., Kreft H., Lee T.M., Jetz W., Ibisch P.L., Nowicki C., Mutke J., Barthlott W., & Tien M.L. (2009) A global assessment of endemism and species richness across island and mainland regions. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 9322–9327.

Lens F., Davin N., Smets Erik, & Arco M. del (2013) Insular woodiness on the canary islands: A remarkable case of convergent evolution. *International Journal of Plant Sciences*, **174**, 992–1013.

Marrero-Gómez M. V., Bañares-Baudet Á., & Carqué-Álamo E. (2005) Viabilidad de las poblaciones del endemismo tinerfeño *Echium auberianum* (Boraginaceae). *Vieraea*, **33**, 93–104.

Marrero-Gómez M. V., Oostermeijer J.G.B., Carqué-Álamo E., & Bañares-Baudet Á. (2007) Population viability of the narrow endemic *Helianthemum juliae* (Cistaceae) in relation to climate variability. *Biological Conservation*, **136**, 552–562.

Mazykin V. V, Platonov A.N., & Dubrovinsky L. (2011) Functional extinctions of bird pollinators cause plant declines. *Science*, **331**, .

Médail F. & Quézel P. (1999) Biodiversity hotspots in the Mediterranean basin: Setting global conservation priorities. *Conservation Biology*, **13**, 1510–1513.

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington DC.

Milligan B.G. & Stubben C.J. (2007) Estimating and Analyzing Demographic Models Using the popbio Package in R. *Journal of Statistical Software*, **22**, issue 11.

Moreno-Saiz J.C. (2008) *Lista Roja de la flora vascular española*. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid.

Moreno-Saiz J.C., Domínguez Lozano F., Marrero-Gómez M. V., & Bañares-Baudet Á. (2015) Application of the Red List Index for conservation assessment of Spanish vascular plants. *Conservation Biology*, **29**, 910–919.

Morris W.F. & Doak D.F. (2002) *Quantitative conservation biology: theory and practice of population viability analysis*. Sinauer Associates, Sunderland.

De Nascimento L., Willis K.J., Fernández-Palacios J.M., Criado C., & Whittaker R.J. (2009) The long-term ecology of the lost forests of La Laguna, Tenerife (Canary Islands). *Journal of Biogeography*, **36**, 499–514.

Niang I., Ruppel O.C., Abdrabo M.A., Essel A., Lennard C., Padgham J., & Urquhart P. (2014) Africa. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change* (ed. by V.R. Barros, C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White), pp. 1199–1265. Cambridge University Press, Cambridge.

Nogales M., Heleno R., Rumeu B., González-Castro A., Traveset A., Vargas P., & Olesen J.M. (2015) Seed-dispersal networks on the Canaries and the Galápagos archipelagos: interaction modules as biogeographical entities. *Global Ecology and Biogeography*, **24**, .

Olesen J.M. (1985) The Macaronesian bird-flower element and its relation to bird and bee oppurtunists. *Journal of the Linnean Society*, **91**, 395–414.

Oostermeijer J.G.B., Vantveer R., Dennijs J.C.M., Veer R.V., & Nijs J. Den (1994) Population-Structure of the Rare, Long-Lived Perennial *Gentiana pneumonanthe* in relation to vegetation and management in the Netherlands. *Journal of Applied Ecology*, **31**, 428–438.

Pierson E.A. & Turner R.M. (1998) An 85-year study of saguaro (*Carnegiea gigantea*) demography. *Ecology*, **79**, 2676–2693.

Pino J., Picó F.X., & De Roa E. (2007) Population dynamics of the rare plant *Kosteletzkya pentacarpos* (Malvaceae): A nine-year study. *Botanical Journal of the Linnean Society*, **153**, 455–462.

R Core Team (2013) R: A Language and Environment for Statistical Computing. Version 3.1.2.

Reyes-Betancort J.A., Santos Guerra A., Guma I.R., Humphries C.J., & Carine M. a. (2008) Diversity, rarity and the evolution and conservation of the Canary Islands endemic flora. *Anales del Jardín Botánico de Madrid*, **65**, 25–45.

Ribeiro P.J. & Diggle P.J. (2015) geoR: Analysis of Geostatistical Data. .

Rodríguez Núñez S., Acevedo Rodríguez A., Oval de la Rosa J.P., Matos J., Rodríguez Martín B., & Rodríguez Martín A. (2004) *Lavatera phoenicea* Vent. *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (ed. by Á. Bañares-Baudet, G. Blanca, J. Güemes, J.C. Moreno, and S. Ortiz), pp. 895. Dirección General de Conservación de la Naturaleza, Madrid.

Salguero-Gómez R., Jones O.R., Archer C.R., Buckley Y.M., Che-Castaldo J., Caswell H., Hodgson D., Scheuerlein A., Conde D. a., Brinks E., de Buhr H., Farack C., Gottschalk F., Hartmann A., Henning A., Hoppe G., Römer G., Runge J., Ruoff T., Wille J., Zeh S., Davison R., Vieregg D., Baudisch A., Altwegg R., Colchero F., Dong M., de Kroon H., Lebreton J.-D., Metcalf C.J.E., Neel M.M., Parker I.M., Takada T., Valverde T., Vélez-Espino L. a., Wardle G.M., Franco M., & Vaupel J.W. (2015) The compadre Plant Matrix Database: an open online repository for plant demography. *Journal of Ecology*, **103**, 202–218.

Schmid S., Schmid V.S., Zillikens A., Harter-Marques B., & Steiner J. (2011) Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees. *Plant Biology*, **13**, 41–50.

Sosa P.A., González-González E.A., González-Pérez M.A., Naranjo-Cigala A., Carqué-Álamo E., & Acevedo Rodríguez A. (2014) Reproductive strategy and ploidy determine the genetic variability of Sorbus aria. *Tree Genetics and Genomes*, **10**, 679–688.

Sperling F.N., Washington R., & Whittaker R.J. (2004) Future climate change of the subtropical North Atlantic:

Implications for the cloud forests of tenerife. *Climatic Change*, **65**, 103–123.

Traveset A., Olesen J.M., Nogales M., Vargas P., Jaramillo P., Antolín E., Trigo M.M., & Heleno R.H. (2015a) Bird–flower visitation networks in the Galápagos unveil a widespread interaction release. *Nature Communications*, **6**, 6376.

Traveset A., Tur C., Trøjelsgaard K., Heleno R.H., Castro-Urgal R., & Olesen J.M. (2015b) Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*, **24**, 1–11.

Tylianakis J.M. (2013) The global plight of pollinators. *Science*, **339**, 1532–1533.

Valido A., Dupont Y.L., & Olesen J.M. (2004) Bird-flower interactions in the Macaronesian islands. Journal of Biogeography, 31, 1945–1953.

Vargas P. (2007) Are Macaronesian islands refugia of relict plant lineages? A molecular survey. *Phylogeography of Southern European refugia* (ed. by S. Weiss and N. Ferrand), pp. 297–314. Springer, Amsterdam.

VVAA (2000) Lista Roja de la Flora Vascular Española. *Conservación Vegetal*, **6**, 1–44.

Westerman P.R., Liebman M., Menalled F.D., Heggenstaller A.H., Hartzler R.G., & Dixon P.M. (2005) Are many little hammers effective? Velvetleaf (Abutilon theophrasti) population dynamics in two- and four-year crop rotation systems. *Weed Science*, **53**, 382–392.

Whittaker R.J. & Fernández-Palacios J.M. (2007) *Island biogeography: ecology, evolution, and conservation.* Oxford University Press, Chippenham.

Síntesis y conclusiones

Síntesis

Esta memoria doctoral ha estudiado una rara especie de la flora de las Islas Canarias para contribuir a responder un conjunto de preguntas sobre la evolución en el archipiélago. Se han combinado el trabajo de campo intenso, técnicas de laboratorio y análisis estadístico para lograr un conocimiento más completo de *Navaea phoenicea* y responder a una parte de los interrogantes sobre su ecología y evolución. A pesar de su singularidad evolutiva dentro de la flora canaria ha permanecido al margen de la investigación y ha sido hasta ahora una especie mal conocida. Existe una notable línea de trabajo en las islas dedicada al conocimiento más profundo de especies señeras de la flora isleña. Así, la investigación realizada en esta tesis incorpora a la higuereta al conjunto de especies simbólicas bien conocidas en la flora amenazada canaria, junto a taxones como *Bencomia exstipulata*, *Lotus berthelotii* o *Echium wildpretii*, entre otras muchas.

Solamente todos los resultados obtenidos en conjunto acerca de la historia natural de *N. phoenicea* permite tener una visión completa de las preguntas formuladas separadamente en cada capítulo. Esta síntesis de la memoria doctoral pretende unir y vincular algunos resultados de cada una de ellos y proponer nuevas líneas de investigación pendientes. Los trabajos desarrollados sobre esta especie, resultan excesivamente novedosos en la metodología utilizada, por su aplicación en conjunto en un marco insular, donde esta tesis sí puede abrir nuevas posibilidades. Los modelos de nicho ecológico, por ejemplo, se utilizan de manera generalizada gracias por un lado a herramientas informáticas accesibles, y por otro a la existencia de información geográfica homogénea, esencialmente topográfica y climática, para a nivel mundial. Esto no es posible en el caso de las islas oceánicas, donde los factores climáticos operan a escalas menores y requieren otra resolución espacial. Esta tesis proporciona nuevas capas climáticas de gran resolución para la isla de Tenerife, pero que se desarrollarán a largo plazo para todas las islas e incorporarán además escenarios de cambio climático. Más allá de los modelos de nicho, parte de los estudios desarrollados toman también en cuenta otros componentes espaciales de algunas de las preguntas estudiadas en la tesis. El análisis con marcadores moleculares, por ejemplo ha aprovechado la georreferenciación de los individuos para establecer el grado de autocorrelación espacial de

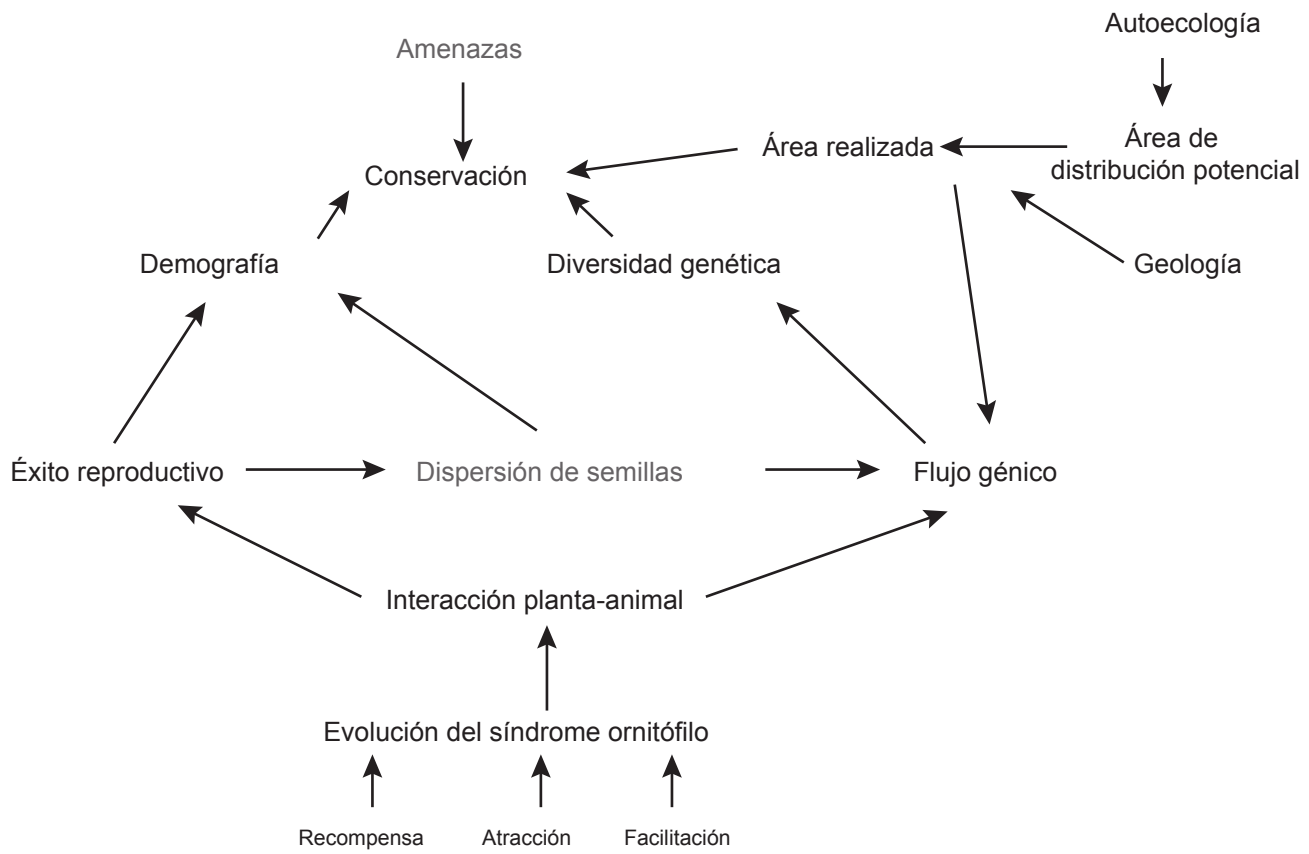


Figura 1. Diagrama de relaciones entre procesos ecológicos y evolutivos estudiados en la memoria. En gris, aquellos procesos pendientes de estudio.

los mismos y establecer la estructura espacial genética a corta distancia, una aproximación poco frecuente en los estudios genéticos en Canarias. Las limitaciones al análisis espacial, concretamente el de las interacciones mutualistas y antagonistas se discuten bajo el epígrafe de las nuevas líneas de investigación.

RESPUESTA EVOLUTIVA AL PAPEL DE LAS AVES PASERIFORMES GENERALISTAS

Los resultados de los capítulos 3, 4 y 5 permiten en su conjunto establecer cuál es la estrategia reproductiva de la *N. phoenicea* y el papel de los polinizadores actuales. El sistema reproductivo hercógamo-dicógamo tradicionalmente reconocido en las Malváceas tiene numerosas excepciones, también en la tribu Malveae. Como adaptación para maximizar el cruzamiento, el mecanismo de fertilización retardada en este caso no es operativo, sino que simplemente la pérdida del polen antes de la autofecundación parece ser suficiente para limitar la funcionalidad de la autofertilización. El desarrollo de los estadios florales estudiado en el capítulo 3 y la dinámica en la producción del néctar floral en el capítulo 5, permiten esclarecer la estrategia del esfuerzo reproductivo de la especie: la corta fase masculina de la flor está acompañada por un mayor esfuerzo en la producción de néctar, mientras en la fase femenina, más larga, el esfuerzo de secreción de néctar es menor. El esfuerzo reproductivo parece por tanto equilibrado entre ambas funciones de la flor hermafrodita. La duración de la fase masculina y femenina y la asignación del esfuerzo reproductivo están por tanto en equilibrio para optimizar el cruzamiento. Aunque estos experimentos no han comprobado el grado de precisión entre flor y animal en el proceso de la polinización, los tres estudios de biología reproductiva permiten establecer que se trata de una especie con adaptaciones funcionales y morfológicas que maximizan el flujo génico a través de un ajuste espacial óptimo en el mutualismo. Una cuarta evidencia potencial es la longevidad del polen comprobada en el capítulo 4. Los granos son inusualmente grandes en esta especie (Pereira-Coutinho *et al.*, en preparación), lo cual puede explicar la existencia de un periodo fértil desde la antesis muy extenso que favorezca el flujo génico.

El hecho de que la especie de estudio sea funcionalmente xenógama la convierte en dependiente de la eficiencia de los visitantes florales. Los parámetros estudiados de eficiencia ofrecen una serie de indicios sobre la evolución de la ornitofilia en *N. phoenicea*, pero no son completamente concluyentes. Los trabajos desarrollados en esta tesis muestran grandes diferencias en la eficiencia como polinizadores de cada una de las aves (capítulo 4). El caso más llamativo es el del herrerillo canario, un ave con gran capacidad para alimentarse de manera oportunista de néctar, tanto en Canarias como en el continente. Su comportamiento disruptivo o antagonista sobre las flores muestra claramente una falta de adaptación a la polinización. En el otro extremo de las aves visitantes, las currucas cabecinegra y capirotada son las especies más eficientes, pero poco frecuentes. En un rango intermedio del espectro de los polinizadores, los mosquiteros son aves muy frecuentes y que muestran una contribución a la polinización algo mayor, pero sobre la base de una menor eficiencia compensada por un mayor índice de visitas. Las diferencias en el comportamiento, pero quizá también de tamaño, apuntan a que el hábito de visita de posarse, que es común en las currucas y los nectarínidos africanos, es mucho más eficiente que el revoloteo de los mosquiteros. De la misma manera, los resultados del capítulo 5, que muestran una distribución asimétrica en la flor de la gran producción de néctar apoyan una adaptación a ese hábito de visita. Se puede concluir que se mantiene una presión de selección por parte de algunos de los paseriformes generalistas actuales, pero los experimentos desarrollados en la tesis no pueden comprobar por sí solos si la presión de selección de los nectarínidos sería mayor. La mayor evidencia a favor de la hipótesis relictual para el síndrome ornitófilo es la tardía colonización de las islas por parte de las aves respecto a la probable edad de *N. phoenicea*. Una evidencia sobre la presión selectiva derivado de nuestros trabajos es la relativa a la dinámica poblacional de la especie. Los parámetros demográficos estimados en el capítulo 6 muestran que en una proporción relativamente baja de años, pero aun así notable, puede darse una tasa de crecimiento finito positiva. El hecho de que se mantenga un cierto éxito reproductivo en los años más benignos proporciona por tanto evidencia sobre el mantenimiento de la presión de selección de las aves paseriformes sobre los caracteres florales.

El resto de los rasgos florales examinados en esta memoria doctoral no describen un caso estrictamente paradigmático del síndrome ornitófilo tradicionalmente descrito (Faegri & Van Der Pijl, 1979; Cronk & Ojeda, 2008), donde se dan tanto adaptaciones para la atracción y la optimización de la eficiencia, como mecanismos para evitar las visitas ilegítimas. No existen barreras de reclamo

o recompensa para la exclusión de ladrones de néctar, ni mecanismos para dotar a los pedicelos florales de una mayor resistencia al peso. Por el contrario sí parecen existir barreras mecánicas para la exclusión de insectos a través de la presencia de células tabulares en los pétalos. Este mecanismo sin embargo no es funcional, puesto que la morfología abierta de la corola y la orientación permiten la entrada de insectos. La estrategia reproductiva de la planta se limitaría a las adaptaciones que permiten la atracción de aves pero sin que existan adaptaciones reales para la exclusión de ladrones de néctar. Es habitual que las plantas ornitófilas secreten volúmenes elevados de néctar. Pero en este caso, el carácter masivo de dicha secreción apunta a que las visitas ilegítimas no interfieren necesariamente en el éxito ni el esfuerzo reproductivo, puesto que no agotan el recurso. Por otra parte, la morfología floral es intermedia entre la actinomorfía, por ser una corola abierta y simétrica y la zigomorfía, por la curvatura de la columna estaminal y la producción del néctar. Los caracteres zigomórficos favorecerían una mayor eficiencia, mientras que los actinomórficos favorecerían la generalización del sistema reproductivo, más necesario en aquellas especies dependientes de la polinización cruzada (Lázaro & Totland, 2014).

EL FLUJO GÉNICO MEDIADO POR POLINIZADORES

La investigación de los últimos años ha permitido comprobar varias hipótesis sobre el efecto en la diversidad genética de los procesos biogeográficos propios de islas (Pérez de Paz & Caujapé-Castells, 2013; Stuessy *et al.*, 2013): la variabilidad genética no es necesariamente menor por efecto de las poblaciones reducidas, ni existen necesariamente cuellos de botella por efecto de los procesos de colonización a larga distancia seguida de aislamiento (Stuessy *et al.*, 2012; García-Verdugo *et al.*, 2015). Además de determinar el grado de diversidad y estructura genética de las subpoblaciones de *Navaea phoenicea*, el trabajo con AFLPs ha permitido determinar la alta frecuencia de hibridación entre grupos genéticos, evidenciando el papel de la movilidad de los polinizadores. Dicha movilidad, es además sorprendentemente alta. Los trabajos en *Isoplexis canariensis* (Rodríguez-Rodríguez *et al.*, 2015) cuantificaron muy poca movilidad para los mosquiteros y herrerillos, entre uno y dos metros entre plantas. Cuando se trata de visitas a los individuos de higueretas, a pesar de estar generalmente agrupados, el patrón de visitas es sensiblemente distinto, ya que ambas especies recorren de media distancias mucho mayores (27 metros los mosquiteros y 11 los herrerillos, capítulo 2). La estrategia común de los visitantes florales suele minimizar el coste energético de la nectarivoría (e.g. Goulson, 2000). La elevada producción de néctar puede contribuir a fomentar mayores distancias de vuelo y visita, disminuyendo los efectos de la estructura genética espacial a pequeña escala. Si la estrategia reproductiva de la especie comprende una adaptaciones para maximizar el flujo génico a través de especies de mayor movilidad, una hipótesis planteada es que la ornitofilia como síndrome adaptativo no sirve solo a la atracción de polinizadores eficientes y a la exclusión de insectos, sino al fomento de un flujo génico mayor. Mientras que la alta diversidad genética encontrada es coherente con la de otras especies en Canarias, la determinación de la estructura espacial se ha analizado para muy pocas especies en el archipiélago y existe por tanto escasa información para la comparación. La comparación directa con *Lavatera acerifolia* se encuentra en preparación. La presencia de información geográfica individualizada permite una primera interpretación sobre la presencia de la existencia de estructura genética espacial a corta y media distancia a través del parámetro *Sp*, a pesar del alcance del flujo génico. Sin embargo, la dispersión de semillas es un elemento que aún queda pendiente y es imprescindible para tener una interpretación completa de la estructura espacial de las poblaciones.

DIFERENCIAS EN LA ESTRUCTURA GENÉTICA Y LA CONSERVACIÓN DE LAS POBLACIONES DE TENO Y ANAGA

A lo largo de los diferentes capítulos se han podido hallar algunas diferencias entre las dos poblaciones, situadas en los macizos de Teno y Anaga. Estas diferencias se dan específicamente en relación a la estructura poblacional, en referencia tanto a la demografía como a la estructura genética. El número total de individuos censados en la población de Anaga es menor que el de Teno, pero sin embargo, el declive poblacional en la primera es ligeramente menor, mientras que la diversidad genética es algo mayor. Sin embargo, la diferencia en la estructura genética espacial a pequeña escala es de mayor magnitud. El aislamiento genético por distancia existe desde el principio en Teno, pero no en Anaga, donde en la distancia más corta no se encontró una autocorrelación significativa en el coeficiente $F_{ij.}$

No hemos encontrado diferencias entre Teno y

Anaga ni en las características físicas del hábitat (clima y topografía), ni en las interacciones mutualistas estudiadas. Sin embargo, los estudios con AFLPs y el monitoreo de la fenología señalan que a pequeña escala sí emergen otras diferencias: mediante estos dos trabajos encontramos un proceso mediante el cual se da la divergencia genética entre subpoblaciones sin existir una barrera geográfica. Es el caso de la subpoblación de Carboneras en Anaga, donde la diferenciación microclimática podría adelantar la floración y contribuir a cierto aislamiento genético.

Una posible causa fundamental para interpretar las diferencias en la estructura espacial genética entre Teno y Anaga, que se encuentra ya en estudio, es el papel del lagarto tizón *Gallotia gallotii* sobre la dispersión de la especie. De manera fortuita, durante el trabajo de campo, se detectaron excrementos de rata común (*Rattus rattus*) y también de lagarto, en ambos casos conteniendo semillas de higuereta. El hallazgo es muy sorprendente dada la manifiesta falta de adaptaciones a la dispersión por aves o lagartos de esta planta. Aunque el papel de estos animales como dispersores de semillas es común en Canarias, este mutualismo se da con especies vegetales de frutos carnosos que resultan de alguna manera atractivos para los frugívoros. *N. phoenicea* por el contrario produce un esquizocarpo seco que *a priori* no resultaría llamativo para un dispersor canario. Sus semillas tienen una testa gruesa y dura que dificulta la germinación. Esta se ve facilitada por la escarificación manual, de manera que una posible ingesta podría disminuir su grosor. Este hallazgo dio pie a un nuevo estudio sobre el efecto en la dispersión de semillas por parte del lagarto tizón. Por una parte, se prospectaron las subpoblaciones de *N. phoenicea* y sus cercanías para detectar fecas de lagarto así como la posible presencia de más semillas de la especie. Después, se comprobó el grado de germinación de parte de las semillas halladas. Por otro lado, se capturaron varios especímenes de lagartos que se mantuvieron en terrarios y se les incorporó a la dieta el mismo número de semillas de *Navaea* (figura 2a). Se comprobó la tasa de germinación de parte de las semillas excretadas, y otra fracción se examinó con microscopía de barrido para comprobar la diferencia en el grosor de la testa respecto a semillas control. Se halló un número escaso de semillas de *N. phoenicea* en los excrementos de *Gallotia*, que eran mucho más escasos en las laderas de umbría donde se encuentran las poblaciones de *Navaea*, que en las áreas más expuestas, donde los excrementos que aparecieron no contenían semillas. En general los lagartos excretaron menos de la mitad de las semillas enteras, pero que presentaron mayor germinabilidad que las semillas control. El grosor de la testa medido sobre semillas que no salieron enteras fue más bajo que el de los controles (figura 2b). Los resultados de este estudio sugieren que, si bien la endozoocoria no es en absoluto frecuente, sí puede facilitar la dispersión de semillas, incluso a larga distancia, por dispersión secundaria. La combinación de una mayor diversidad genética en Anaga y un patrón diferente de estructura genética espacial a pequeña, junto a una mayor tasa de crecimiento finito, quizás puedan deberse a la superior actividad de *Gallotia galloti* sobre *Navaea* en ese macizo. Asimismo, la dispersión a mayores distancias que ofrece la zoocoria puede también explicar el alto grado de intercambio genético entre las subpoblaciones.

RETOS PARA LA CONSERVACIÓN

Desde el punto de vista de la biología de la conservación, esta memoria doctoral examina varios factores que afectan a la conservación de la especie de estudio: la extensión de su presencia (capítulo 1), el número de efectivos, las tendencias poblacionales y la probabilidad de extinción (capítulo 6). También confirma el éxito reproductivo mediado por los actuales paseriformes generalistas. Así, se confirma el carácter amenazado de *Navaea phoenicea* por la baja superficie ocupada por la planta y las tendencias demográficas proyectadas. Aunque el examen de la dinámica poblacional no profundiza en los factores de riesgo, sí detecta una asociación muy clara entre el crecimiento finito de la población y la precipitación anual. La contribución de otras amenazas no queda clara. La herbivoría, tanto por parte del pastoreo como de las especies introducidas (muflones, ratas, conejos) afecta de manera inequívoca a la vegetación en el archipiélago. Los análisis preliminares expuestos en el capítulo 6 apuntan también a un papel de los herbívoros en la supervivencia de las plántulas. De confirmarse, el tamaño de las poblaciones de *Navaea* sería mayor en ausencia de herbivoría. El nicho climático de la especie se mantendría como factor limitante para la ampliación de la extensión de las poblaciones. La pregunta a resolver es qué contribución a la dinámica poblacional es atribuible a cada uno de los dos factores, clima y herbivoría, por lo que es necesario efectuar un estudio más detallado de los vallados de exclusión.

La tendencia negativa de las poblaciones se manifiesta o se proyecta a largo plazo dada la alta supervivencia de los individuos reproductores, a la que el análisis de elasticidad

atribuye el mayor peso en los resultados. Las medidas de conservación focalizadas a proteger estos individuos incluirían una protección integral de sus hábitats, como se discutía en el capítulo 6, y la preservación frente a eventos catastróficos que puedan acabar con las poblaciones. Esta protección tiene que ir más allá de la preservación del hábitat en relación a la planificación territorial. La intervención en la gestión pasa por varias acciones, como son también vigilar y controlar, y en su caso erradicar, las especies exóticas invasoras. Esto es importante por dos razones: la primera es la competencia territorial con la flora autóctona (Capistrano, 2005); la segunda es la disrupción de alóctonas en las redes mutualistas, que pueden competir por la atracción de polinizadores y frugívoros y comprometer el éxito reproductivo de las especies nativas (Traveset & Richardson, 2006). La conservación del hábitat pasa también por el mantenimiento de dichas redes mutualistas y la suficiente abundancia de poblaciones de los visitantes florales. *Navaea* puede ser una especie importante en las áreas rupícolas del bosque termófilo, dado que es visitada por un alto número de especies. De ellas, tres son polinizadores efectivos, lo que puede proporcionar una cierta tolerancia frente a la extinción de especies. El carácter semirupícola requiere también focalizar la atención hacia los procesos erosivos y de destrucción del suelo que pueden ser más intensos en las zonas de elevada pendiente, máxime en áreas sometidas a laboreo agrícola y ahora abandonadas. Finalmente, es necesario reforzar la disponibilidad de germoplasma con semillas procedentes de los ocho grupos genéticos

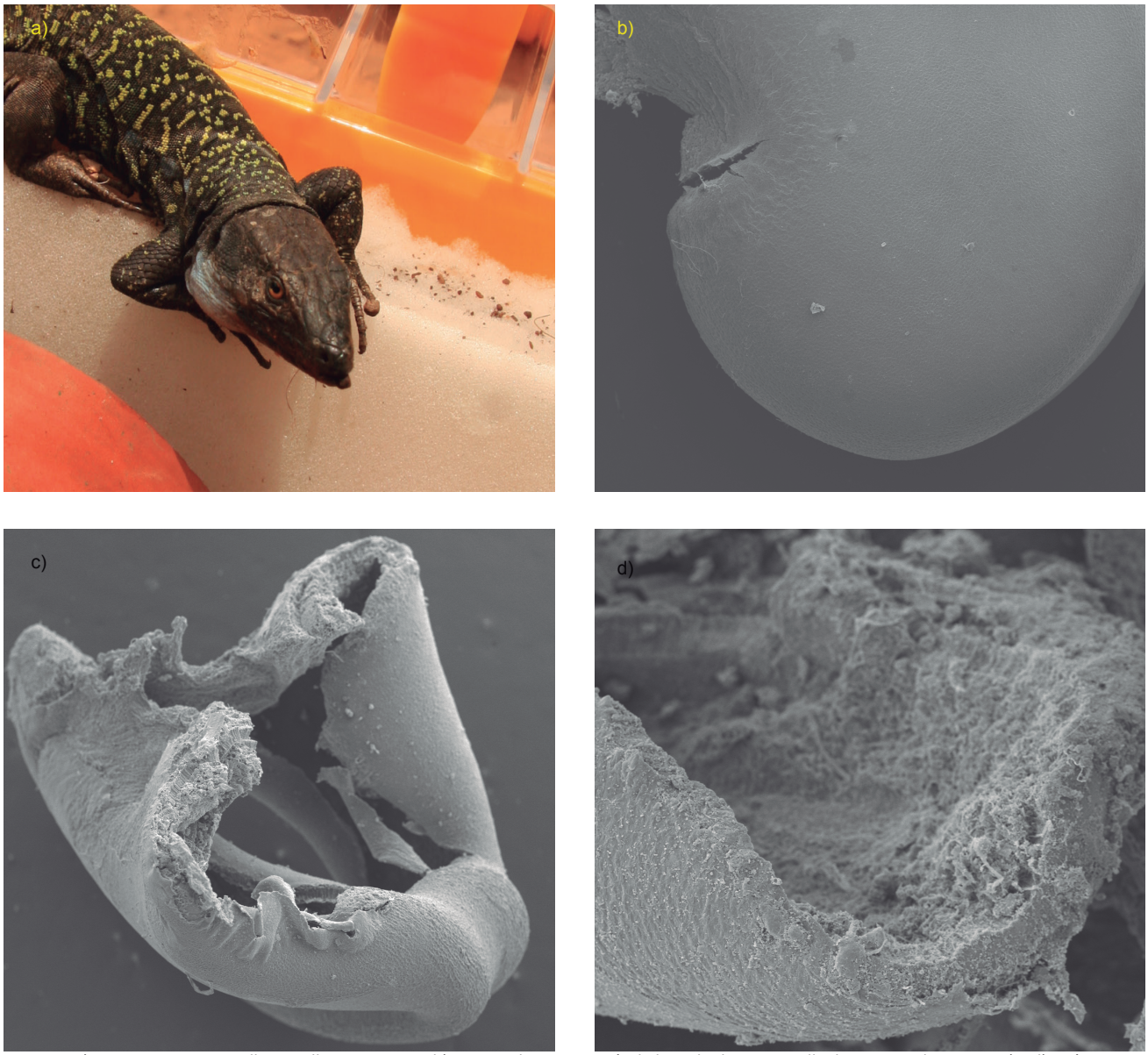


Figura 2. a) Experimento con *Gallotia galloti* en terrario. b) Imagen de microscopía de barrido de una semilla de Navaea phoenicea. c) y d) Imágenes de microscopía de barrido de una semilla digerida por un lagarto tizón en cautividad.

identificados por los AFLPs. Es remarcable mencionar las diferencias potenciales entre Teno y Anaga en cuanto al manejo del territorio, dado que la protección legal en uno u otro macizo ha cambiado recientemente. Inicialmente ambas áreas quedan protegidas bajo las Directivas de Hábitats y Aves por una parte, y por otro bajo la figura de Parque Rural, propia del Gobierno de Canarias. Los planes de manejo y gestión obligatorios para las áreas de la Red Natura 2000 quedan supeditados, como práctica habitual, a los planes de uso y gestión de otras figuras de protección si las figuras de protección se solapan. En el caso de la figura de Parque Rural, se busca una convivencia equilibrada en el uso del territorio entre la conservación de la naturaleza y las actividades tradicionales de dichas áreas, principalmente las agropecuarias. Si bien se fomenta la conservación del paisaje y consecuentemente de los hábitats, la figura de Parque Rural no garantiza necesariamente la protección frente a planificaciones del territorio con consecuencias dañinas. Además, la vocación de compatibilizar actividades agropecuarias en un contexto de conservación, tiene como consecuencia lógica la persistencia de procesos de herbivoría por parte del ganado, sobre todo cabras, por todo el territorio. Recientemente el macizo de Anaga ha sido reconocido como reserva de la Biosfera por parte de la UNESCO, lo que puede ser una oportunidad para reforzar las medidas y recursos de protección para los hábitats del área.

Por último, se pueden apoyar algunas medidas complementarias en relación al refuerzo poblacional que ya proponen Rodríguez-Núñez *et al.* (2004) y que serían de manera general bastante efectivas en Canarias (Marrero-Gómez *et al.*, 2003). Las áreas identificadas como topográfica y climáticamente idóneas por los modelos de nicho se limitan esencialmente a las áreas de las paleoislas, pero asignan una alta idoneidad también a varios barrancos del norte de la isla, dibujando un área potencial discontinua pero no tan fragmentada como la que se da actualmente en la isla. La idea de las posibles poblaciones puente o intermedias se discute en los capítulos 1 y 2, dado que la existencia de una población entre las dos paleoislas facilitaría el flujo génico entre núcleos. Una propuesta ya proyectada es la validación directa de los modelos de nicho mediante la plantación de poblaciones experimentales en esos barrancos del centro de la costa norte.

NUEVAS LÍNEAS DE INVESTIGACIÓN

Del trabajo de esta tesis doctoral, y las discusiones que la han acompañado, surge una serie de propuestas de investigación que se van exponiendo a continuación.

El desarrollo de los modelos de nicho ha permitido definir una metodología para obtener capas climáticas que pueden desarrollarse también para el resto de las islas, y que quedarán a libre disposición para realizar modelos con otras especies. Además, la aproximación para reducir la escala de los escenarios de cambio climático del quinto informe del IPCC permite proyectar los impactos del cambio climático sobre la distribución de las especies a alta resolución. El trabajo de modelización realizado con *Navaea* permite además que el catálogo de variables ofrecidas sea más amplio que los conjuntos de datos más conocidos como Worldclim (Hijmans *et al.*, 2005), que no tienen la resolución adecuada para escenarios pequeños como las islas; en este caso, se incorporan variables de radiación potencial, evapotranspiración potencial, y un modelo nivológico para las Cañadas del Teide (figura 3).

El empleo de marcadores moleculares supone un reto al tratarse de una especie poliploide, pero la aplicación de microsatélites cloroplásticos pueden ser de gran utilidad para establecer patrones de dispersión más precisos, puesto que el trabajo desarrollado hasta ahora se ha basado en la asunción de que la planta madre es el individuo adulto más próximo. Además, el uso de secuencias de genes puede apoyar las evidencias halladas mediante AFLPs y avanzar hacia una datación de la disyunción.

Las evidencias obtenidas que sustentan la hipótesis

Figura 3. Flor de *Lavatera assurgentiflora*.

relicta de la evolución del síndrome ornitófilo permite proseguir dos líneas de investigación, una sobre la eficiencia de las interacciones, y otra sobre la evolución de los caracteres florales. Respecto a la primera, algunos de los estudios necesarios son la medición de la precisión en la polinización por parte de los visitantes florales *sensu* Armbruster (2009). También es necesario profundizar en las posibles adaptaciones del polen a la polinización ornitófila, y estudiar el efecto tanto de la densidad de polinizadores sobre la frecuencia, como incorporar la cuantificación de la variabilidad espacial en las relaciones mutualistas y antagonistas. La ejecución de nuevas acciones experimentales adquiere mucho más sentido si se da la posibilidad de realizar experimentos también confrontando la interacción de los nectarínidos africanos para estudiar el grado de eficiencia de este grupo respecto a los paseriformes generalistas. Los experimentos de manipulación de rasgos florales o el trabajo con polinizadores en condiciones controladas han permitido en otras ocasiones determinar los cambios en las preferencias del visitante por determinados rasgos en la flor (Dudash *et al.*, 2011; Lunau *et al.*, 2011), así como grandes cambios poco esperados en el comportamiento (Janecek *et al.*, 2011). Existe igualmente otra pregunta interesante sin responder, sobre la respuesta adaptativa de los abejorros canarios (*Bombus canariensis*) a los rasgos florales del elemento ornitófilo Macaronésico. La hipótesis de Skorupski *et al.* (2007) de que la mayor proporción de especies con flores rojas en archipiélagos como el canario ha fomentado una mayor sensibilidad de los abejorros insulares a los espectros lumínicos de alta frecuencia aún carece de soporte empírico. En el caso concreto de Canarias, es necesario en primer lugar medir la sensibilidad de los fotorreceptores de *Bombus canariensis* y cómo han variado respecto a los abejorros continentales, y en segundo lugar comprobar experimentalmente las respuestas a la presencia de flora ornitófila Macaronésica.

La caracterización de los rasgos florales ofrece igualmente varias oportunidades para proseguir los estudios. En relación a las propiedades relacionadas con el néctar, existen dos características obvias que es necesario evaluar: por una parte, la caracterización de aminoácidos presentes en el néctar es fundamental para comprobar el tipo de recompensa. Hasta el momento es un elemento desconocido para el elemento ornitófilo Macaronésico, así como tampoco está bien caracterizado para las Malváceas. Por otro lado, la dicotomía clásica en la preferencia por un tipo u otro de azúcares (disacáridos frente a monosacáridos) no está en realidad comprobada, ya que en las familias de paseriformes generalistas que han incorporado el néctar de plantas macaronésicas a la dieta no se ha comprobado la capacidad para digerir disacáridos, aunque se puede inferir algunos casos. La falta de caracterización de las propiedades del néctar en las Malveas también abre una puerta para la investigación de la evolución de estas propiedades en las especies de la tribu. El capítulo 5 enumera las especies ya estudiadas.

La evolución de los caracteres florales en Malveas afecta también a otro rasgo importante como es el color. Sobre *Navaea phoenicea* el estudio actual está limitado a la percepción visual del espectro reflejado por las piezas florales de acuerdo al contexto natural. Aunque los resultados permiten hacer una interpretación del patrón de color, es necesario hacer una caracterización

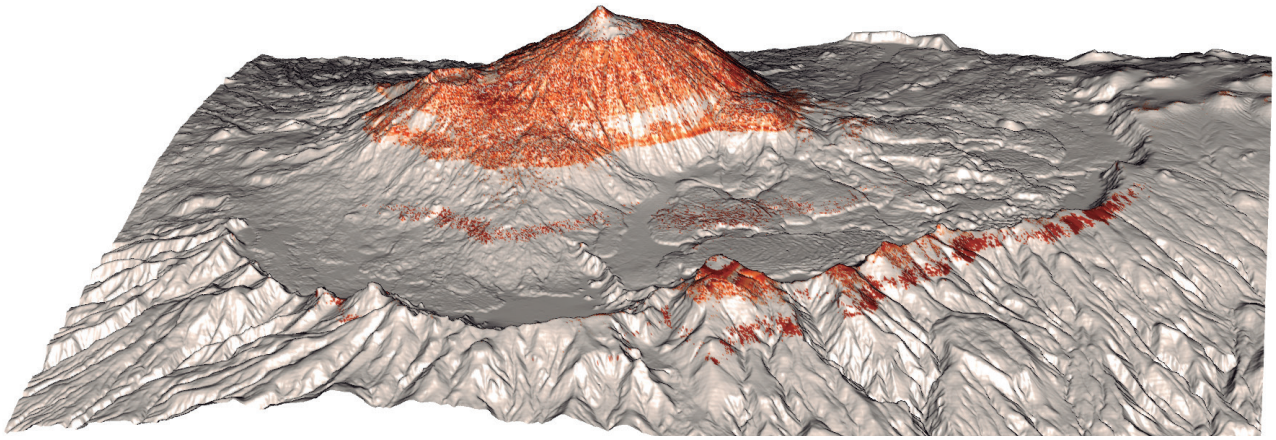


Figura 4. Modelo nivológico para las Cañadas del Teide en el escenarios de cambio climático MIROC-RCP 6.0 elaborado para el archipiélago Canario a escala de 5 metros.

más exhaustiva, dado el color especialmente llamativo y de las flores de esta especie respecto a las malvas en general. Por tanto, por una parte es necesario identificar y aislar los pigmentos de los colores que causan dichos patrones de reflectancia, y por el otro determinar las rutas metabólicas de los pigmentos. Esto es extensible además al linaje de las Malveas, de manera que se pueda conocer tanto la composición en pigmentos de cada especie como la filogenia de los genes reguladores de las rutas metabólicas que los producen. La línea evolutiva de esta tribu y especies cercanas como *Navaea* también da pie al estudio de la evolución del sistema reproductivo, del cual solo se conoce la evolución de pocas especies. Hasta la fecha, es el género *Lotus* es el que cuenta con un conocimiento más complejo de la evolución de los caracteres florales (Ojeda *et al.*, 2012, 2013).

En relación a la evolución de la biología reproductiva, también es reseñable la oportunidad brindada por otra especie insular, *Lavatera assurgentiflora* de las Islas del Canal en California (figura 4). A diferencia de *Navaea*, sí se está filogenéticamente emplazada en la tribu Malveae, y guarda ciertas similitudes con las dos malvas canarias: se trata de endemismos insulares (en este caso de una isla continental), de porte arbóreo y cuyas flores también son visitadas por aves, en su caso mayoritariamente colibríes (*Selasphorus sasin*, observación personal). Se trata de una especie con poca información sobre su biología, por lo cual el posible cambio en el síndrome de polinización es desconocido, pero puede aportar nuevos datos sobre cómo ha evolucionado la presión de selección sobre los caracteres florales en la familia de las Malváceas. Una de las aproximaciones más útiles desarrolladas para la ecología de la polinización en los últimos tiempos es la aproximación Evo-Devo (Sapir, 2009; Glover *et al.*, 2015), dadas las adaptaciones que presenta esta especie. Más específicamente, la evo-devo puede caracterizar los siguientes rasgos: la exclusividad de los receptáculos para albergar el néctar, la elevada presencia de tricomas nectaríferos así como su distribución radial en la flor, y la zigomorfía provocada por la curvatura de la columna estaminal (Zhang *et al.*, 2010). La caracterización de los genes que controlan dicho desarrollo y su expresión en las fases florales serán de extrema utilidad para el estudio de la evolución de los caracteres florales y la selección de los mismos por parte de los polinizadores.

CONCLUSIONES FINALES

El archipiélago canario es uno de los sistemas insulares más reconocidos en todo el mundo por las ciencias naturales, y esta tesis pone en relieve de nuevo el papel de laboratorios naturales que ofrecen las islas, en particular Canarias. En el contexto del conocimiento actual de la biogeografía insular, nuestro trabajo contribuye a reivindicar las aproximaciones integradas a la biología de los organismos para comprender aspectos concretos de la evolución de plantas en el contexto isleño. En el caso de *Navaea phoenicea*, estos aspectos son: adaptaciones florales mantenidas por la presión de selección de aves generalistas en un medio insular, una estructura genética determinada por las características geológicas de una isla volcánica, y una serie de amenazas impulsadas por el cambio global en islas. Medio siglo después de la Teoría de McArthur y Wilson (1967), la biogeografía insular ha impulsado un ingente conocimiento alrededor de la diversidad biológica de las islas. Pero también reivindicamos aquí otro número de singularidades evolutivas de la biota isleña, que son consecuencia de los procesos evolutivos que surgen en un entorno aislado. Así, este trabajo sirve igualmente para poner en valor otros elementos: el primero de ellos, la aplicación de nuevas herramientas de estudio de la singularidad de la biota de las islas oceánicas. Y el segundo, el conocimiento íntegro de las especies para entender por completo las causas de su declive.

REFERENCIAS

Armbruster W.S., Pélabon C., Hansen T.F., & Bolstad G.H. (2009) Macroevolutionary patterns of pollination accuracy: A comparison of three genera. *New Phytologist*, **183**, 600–617.

Capistrano D. (2005) *Ecosystems and human well-being: Multiscale assessments : findings of the Sub-global Assessments Working Group of the Millennium Ecosystem Assessment*. Island Press, Washington, DC.

Cronk Q. & Ojeda D.I. (2008) Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, **59**, 715–727.

Dudash M.R., Hassler C., Stevens P.M., & Fenster C.B. (2011) Experimental floral and inflorescence trait manipulations affect pollinator preference and function in a hummingbird-pollinated plant. *American Journal of Botany*, **98**, 275–82.

Faegri K. & Van Der Pijl L. (1979) *The principles of pollination ecology*. Elsevier, Oxford.

García-Verdugo C., Sajeva M., La Mantia T., Harrouni C., Msanda F., & Caujapé-Castells J. (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. *Molecular Ecology*, **24**, 726–741.

Glover B.J., Airoldi C.A., Brockington S.F., Fernández-Mazuecos M., Martínez-Pérez C., Mellers G., Moyroud E., & Taylor L. (2015) How have advances in comparative floral development influenced our understanding of floral evolution? *International Journal of Plant Sciences*, 000–000.

Goulson D. (2000) Why do pollinators visit proportionally fewer flowers in large patches? *Oikos*, **91**, 485–492.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Janecek Š., Patácová E., Bartoš M., Padyšáková E., Spitzer L., & Tropek R. (2011) Hovering sunbirds in the Old World: Occasional behaviour or evolutionary trend? *Oikos*, **120**, 178–183.

Lázaro A. & Totland Ø. (2014) The influence of floral symmetry, dependence on pollinators and pollination generalization on flower size variation. *Annals of Botany*, 157–165.

Lunau K., Papiorek S., Eltz T., & Sazima M. (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *The Journal of Experimental Biology*, **214**, 1607–12.

Marrero-Gómez M. V., Bañares-Baudet Á., & Carqué-Álamo E. (2003) Plant resource conservation planning in protected natural areas: an example from the Canary Islands, Spain. *Biological Conservation*, **113**, 399–410.

McArthur R.A., Wilson E.O., MacArthur R.H., & Wilson E.O. (1967) *The theory of island biogeography*. Princeton University Press, Princeton.

Ojeda D.I., Santos-Guerra A., Caujapé-Castells J., Jaén-Molina R., Marrero-Rodríguez Á., & Cronk Q. (2012) Comparative micromorphology of petals in macaronesian lotus (Leguminosae) reveals a loss of papillose conical cells during the evolution of bird pollination. *International Journal of Plant Sciences*, **173**, 365–374.

Ojeda D.I., Santos-Guerra A., Oliva-Tejera F., Valido A., Xue X.., Marrero-Rodríguez Á., Caujapé-Castells J., & Cronk Q. (2013) Bird-pollinated Macaronesian *Lotus* (Leguminosae) evolved within a group of entomophilous ancestors with post-anthesis flower color change. *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 193–204.

Pérez de Paz J. & Caujapé-Castells J. (2013) A review of the allozyme data set for the Canarian endemic flora: causes of the high genetic diversity levels and implications for conservation. *Annals of Botany*, **111**, 1059–73.

Rodríguez Núñez S., Acevedo Rodríguez A., Oval de la Rosa J.P., Matos J., Rodríguez Martín B., & Rodríguez Martín A. (2004) *Lavatera phoenicea* Vent. *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (ed. by Á. Bañares-Baudet, G. Blanca, J. Güemes, J.C. Moreno, and S. Ortiz), pp. 895. Dirección General de Conservación de la Naturaleza, Madrid.

Rodríguez-Rodríguez M.C., Jordano P., & Valido A. (2015) Hotspots of damage by antagonists shape the spatial structure of plant-pollinator interactions. *Ecology*, **96**, 2181–2191.

Sapir Y. (2009) Pollination genetics: Using molecular genetic underlying floral traits to study pollination ecology in an evolutionary context. *Israel Journal of Plant Sciences*, **57**, 141–149.

Skorupski P., Döring T.F., & Chittka L. (2007) Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology*, **193**, 485–94.

Stuessy T.F., Takayama K., López-Sepúlveda P., & Crawford D.J. (2013) Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society*, **174**, 276–288.

Stuessy T.F., Takayama K., & Sepúlveda P.L. (2012) Founder effects are invisible in endemic species of oceanic islands. *Journal of Biogeography*, **39**, 1565–1566.

Traveset A. & Richardson D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology and Evolution*, **21**, 208–216.

Zhang W., Kramer E.M., & Davis C.C. (2010) Floral symmetry genes and the origin and maintenance of zygomorphy in a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences*, **107**, 6388–6393.

CONCLUSIONES

1. La modelización de nicho muestra que la distribución de *Navaea phoenicea* está delimitada factores climáticos condicionados por la topografía, concretamente la evapotranspiración. Esta distribución limita a la especie a las paleoislas de Teno y Anaga, donde se da la idoneidad topoclimática. La evidencia aportada por los modelos de nicho ecológico basada en el consenso de 4 algoritmos concluye que la disyunción actual puede ser explicada por la existencia de deslizamientos y vulcanismo (0.15 MA), más que a una reciente extirpación producida por perturbación antrópica. No obstante

2. La diversidad genética de *Navaea phoenicea* muestra valores por encima de los esperados para una especie arborescente, de poblaciones fragmentadas y rara con poblacion de 800 individuos. La poliploidía y la fecundación cruzada, que contribuye al mantenimiento de un flujo génico elevado, son factores que pueden explicar su mayor diversidad genética.

3. Existe una estructura genética geográfica a escala de toda la isla con ocho grupos genéticos bien diferenciados en las poblaciones de Teno y Anaga. Existe asimismo estructura espacial a distancias más cortas. La distribución espacial de los fenotipos de AFLPs a pequeña y gran escala está en equilibrio entre la dispersión de las semillas por barocoria y la larga distancia que recorre el polen con *Phylloscopus canariensis* como vector principal.

4. La estructura genética de *Navaea phoenicea* apoya la hipótesis del efecto de la geología como barrera genética en islas. Aun así, el flujo génico mediado por polinizadores es mayor que los límites de la mayoría de las subpoblaciones, de manera que permite el intercambio génico entre los distintos núcleos.

5. La fenología es un factor determinante para explicar el menor flujo génico interpoblacional y la mayor diferenciación genética en la subpoblación de Carboneras. Este grupo presenta una fenología adelantada como consecuencia del microclima local.

6. En cuanto sus sistema reproductivo, *Navaea phoenicea* es una especie totalmente autocompatible, pero el mecanismo de fecundación retardada para asegurar la reproducción por autogamia no es funcional en la especie. Este hecho, que no es infrecuente en Malváceas, se interpreta como una adaptación para favorecer la fecundación cruzada y asegurar una mayor diversidad genética frente a un mayor éxito reproductivo. El patrón temporal de secreción de néctar, de hasta 0.5 ml por flor, apoya esta hipótesis. El esfuerzo es diferente entre la fase masculina y la fase femenina, compensando la duración diferente.

7. Se confirma la eficiencia como polinizadores de tres de las cuatro especies de paseriformes que visitan la especie de estudio (*Sylvia atricapilla*, *Sylvia. melanocephala* y *Phylloscopus canariensis*). Existen no obstante diferencias significativas en los parámetros cualitativos y cuantitativos de su eficiencia. Estas diferencias provienen del comportamiento como visitante propio de cada especie. La cuarta especie, *Cyanistes teneriffae* presenta un comportamiento disruptor sobre la flor, ejerciendo por tanto una falta de presión de selección por parte de esta especie. Por el contrario, los hábitos de visita de las demás aves sí permiten el éxito reproductivo de las flores.

8. Las diferencias de comportamiento entre *Sylvia melanocephala*, *Sylvia atricapilla* y *Phylloscopus canariensis* implican desiguales eficiencias de las visitas. El hábito más eficiente es el perching, atribuido sobre todo a las currucas. Tanto la morfología de la columna estaminal como la asimetría en la producción del néctar indican una adaptación de la flor para favorecer este hábito. Al compartir nectarínidos africanos y currucas este comportamiento de visita, no existen evidencias concluyentes sobre el origen del síndrome ornitófilo en esta especie. No obstante, los tiempos de colonización de Canarias por parte de los paseriformes inferida a partir de la filogenia molecular confirman el origen antiguo del síndrome.

9. Los cálculos del espectro de polinizadores permiten identificar las diferencias en los componentes de la eficiencia de los polinizadores y establecer comparaciones entre especies. Los mosquiteros parecen ser mucho menos eficientes con *Navaea phoenicea* que con *Isoplexis canariensis*. (0.028 versus 0.19). No obstante, los criterios para incluir medidas sobre los componentes del espectro deben revisarse.

10. Las flores de *Navaea phoenicea* presentan un estímulo visual conspicuo no solo para las aves. La especie no ha eliminado la reflectancia en el espectro azul y ultravioleta en los pétalos. Existe un pico de reflectancia azul-ultravioleta del 20% en los pétalos, aunque no en los recéptaculos ni la columna estaminal. Este patrón permite una detectabilidad óptima para los insectos, para los insectos, para los cuales se presenta una fuente abundante y predecible de néctar en otoño. Este pico de reflectancia azul está presente de manera generalizada en las Malveas.

11. Además de las aves, tres especies autóctonas (*Pararge xiphioides*, *Ancistrocerus haematodes* y *Bombus canariensis*) y una especie introducida (*Apis mellifera*) son visitantes ilegítimas e irregulares de las flores. La falta de adaptaciones visuales para excluir insectos ilegítimos puede deberse a que la alta secreción de néctar previene una reducción del éxito reproductivo. No obstante, sí existe un mecanismo, aparentemente no funcional, de exclusión de insectos, basado en la morfología de las células epidérmicas de los pétalos.

12. El patrón de secreción de néctar en *Navaea phoenicea* es característico de las especies ornitófilas, con volúmenes secretados muy elevados de néctar diluido. Existe variabilidad en la producción a lo largo de los estadios florales y variabilidad temporal ligada a la disponibilidad de recursos hídricos. Las flores responden al estímulo de la extracción de néctar manteniendo el ritmo de secreción. Se confirma el carácter estable de la composición en azúcares, a base de glucosa y fructosa exclusivamente.

13. Se confirma el rasgo de arborescencia insular. La existencia de anillos de crecimiento confirma la presencia de lignificación de los tejidos. La máxima longevidad inferida a partir de los anillos son 32 años, mientras que en promedio la edad de los individuos medidos era de 18 años.

14. El estado de conservación estimado a partir del censo anual muestra un declive paulatino de las poblaciones de la especie de estudio. Los años benignos en precipitación favorecen el crecimiento de las poblaciones, con tasas de crecimiento finito de hasta 1.032. La tasa de crecimiento negativa está atenuada por la longevidad de los individuos reproductores. La influencia de la herbivoría requiere estudios más extensos para confirmar el efecto sobre la supervivencia de las plántulas. La disminución de las precipitaciones por efecto del cambio climático es un factor de riesgo dado que se predice una disminución de las precipitaciones.

15. Sobre la base de los criterios de la UICN de la extensión de la presencia (criterio B1), la probabilidad de extinción (criterio A3) y la probabilidad de extinción (criterio E), la especie debe permanecer catalogada como En Peligro. Bajo el criterio B2 la especie sería considerada como Vulnerable. Las recomendaciones son: protección integral del hábitat, estudios extensos del efecto de la herbivoría, refuerzo poblacional y la migración altitudinal asistida.